Rethinking articular cartilage regeneration based on a 250-year-old statement

Jos Malda[1,2]*, *Jürgen Groll*[3] *and P. René van Weeren*[2]

Cartilage has a limited healing capacity; however, studies into the basic biological characteristics, formation and structural maintenance of the cartilage collagen network are providing explanations for the failure of current therapeutic approaches, urging us to rethink our approach to the regeneration of articular cartilage.

> "we cannot restore the biomechanical properties of cartilage via traditional regenerative medicine approaches"

The musculoskeletal system provides shape and stability to the body and enables motion. As an avascular and aneural component of this system, articular cartilage has an almost exclusively biomechanical function. The word 'biomechanics' comes from the Ancient Greek terms for 'life' and 'mechanics' and refers to the study of the mechanical principles of living organisms; in other words, how living tissues deal with mechanical demands. In mechanical terms, the strength or carrying capacity of any structure is determined by the mechanical characteristics of the components of the structure and the spatial architecture of these components. This principle is of particular importance for articular cartilage, given its biomechanical function in the body.

In 1743, William Hunter stated "If we consult the standard Chirurgical Writers from Hippocrates down to the present Age, we shall find, that an ulcerated Cartilage is universally allowed to be a very troublesome Disease; that it admits of a Cure with more Difficulty than a carious Bone; and that, when destroyed, it is never recovered"[1]. This centuries-old observation is as true today as it was in Hunter's time, unlike many other medical observations made in the mid-18th century. Clinically, the introduction of metal implants in the middle of the last century has had an enormous effect on the quality of life of many individuals with joint disease, as these devices can usually restore biomechanical function to the joint for up to 20 years. However, such treatment does not result in the restoration of articular cartilage.

In the past few decades, extensive efforts have been made to achieve functional repair or even complete regeneration of articular cartilage. However, these attempts have consistently failed, despite many of them initially resulting in the gradual formation of a cartilage-like tissue. The reason for the lack of progress in cartilage regeneration might, at least in part, be attributable to a focus on the cell biology aspects, rather than on the mechanical aspects, of the problem. Additionally, a lack of knowledge about the basic biology, formation and maintenance of the biomechanically decisive features of articular cartilage — the components and the architecture of its extracellular matrix — is an important issue.

In 1925, Alfred Benninghoff discovered that the collagen in hyaline cartilage is organized into an arcade-like structure[2]. The 'pillars' of these arcades are firmly anchored in a layer of calcified cartilage and their actual arches are linked to tangential collagen fibres running parallel to the joint surface in the lamina splendens. This knowledge enabled a better understanding of how the entire composite structure of hydrophilic proteoglycans interspersed in a tough collagen network provides the desired combination of strength and resilience needed for the proper function of articular cartilage through the interaction of mechanical and electrostatic forces[3].

Many attempts at regenerating cartilage have produced hyaline-like tissue in vitro; in these techniques a variety of cells were able to produce copious amounts of proteoglycans and type II collagen[4]. However, when tested in vivo in large animal models, none of these techniques could restore the architecture of the collagen network, and instead formed fibrocartilaginous repair tissue[5], which explains their functional failure.

In the early 1990s, important work on collagen metabolism[6] showed that type II collagen from healthy mature individuals had extremely long turnover times, in the order of hundreds of years. Another elegant study[7] based on carbon dating that used the fact that the level of radioactive carbon in the atmosphere has fluctuated considerably as a result of man-made nuclear activity since the Second World War produced irrefutable evidence that the metabolic turnover of the collagen network in cartilage is indeed nil in mature individuals, irrespective of whether or not a person is affected by articular disease, such as osteoarthritis.

This inherent incapacity of the network of type II collagen fibrils to repair or re-form within any biologically relevant timeframe and, hence, the inability to restore the architecture of articular cartilage, must be

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> a shift in focus is urgently needed regarding the development of regenerative medicine approaches for cartilage

considered. This incapacity means that the proven ability of cells to produce and secrete the correct matrix components is not enough for long-term functionality, as biomechanically indispensable architectural structures are not formed. Hence, the prevailing paradigm of regenerative medicine, the aim of which is to use our body's own resources to regenerate, rather than to replace or to repair tissue[8], does not apply to articular cartilage in mature individuals.

Accepting this insight means accepting that we cannot restore the biomechanical properties of cartilage via traditional regenerative medicine approaches and explains why we have thus far not been able to reproduce the healthy native tissue in vivo, either anatomically or functionally. This situation, which is still largely ignored in the field, implies that the classic tissue engineering approach[9] that has been pursued for cartilage for the past 25 years will never be able to provide a long-term functional solution and must be abandoned. A radical change in focus for the regeneration of articular cartilage is, therefore, required if we want to improve on Hunter's sombre prognosis.

We are aware of many methods for cartilage repair that give good, or even excellent, clinical results. For example, allograft transplantation has produced promising results because the required collagen structure is maintained in the transplanted material, as have bioartificial implants that provide this structure; however, integration of grafts and implants into the surrounding tissue remains a challenge[4]. From an engineering point of view, it is the increasingly sophisticated techniques available to researchers (such as bioprinting) that have contributed to progress in many aspects of cartilage regeneration[4]. However, to date, none of these techniques addresses the important aspect of reconstruction of the collagen architecture, which might be owing to an insufficient ability to replicate the orientation and fibre diameter of native collagen. The interaction of biology and mechanics to determine the function of articular cartilage conceptually leads to two distinct avenues that might be explored. We hypothesize that exploring these avenues, either separately or in a combined approach, might break through the current deadlock.

First, acknowledging the fact that the body lays down a definitive and life-long immutable structural element of cartilage in the juvenile phase of life that, unlike almost any other tissue, does not renew itself at regular intervals, could lead to the concept of manufacturing constructs that also contain an immutable part. In those constructs, long-term (non-degradable) structure-giving materials could be combined with regenerative components, such as cell-loaded or cell-instructive biodegradable hydrogels, thereby forming a favourable environment for the formation of articular cartilage tissue. The long-lasting structural element would provide sufficient biomechanical resistance to guarantee functionality from the onset of implantation, thereby enabling the optimal formation of neo-tissue that would, as in native cartilage, lubricate the joint and protect the structural element against wear and tear.

A second approach relies on the observation that the natural arcade-shaped collagen structures that provide the mechanical resilience of the cartilage are formed during the late fetal and early juvenile phases of life[10]. Partial restoration of the microenvironment prevalent in these stages of life (which includes the appropriate cytokine and growth factor profile and targeted mechanical loading) might be achieved by the use of rejuvenated or induced pluripotent stem cells, which have the potential to mimic this juvenile milieu. This process could be supported and accompanied by biomaterials that transiently mimic the structural features of cartilage.

Taken together, we propose that a shift in focus is urgently needed regarding the development of regenerative medicine approaches for cartilage. Unravelling the mechanisms by which the collagen structure of cartilage is initially formed will undoubtedly be a decisive breakthrough in attempts to restore it at later stages, and might have implications beyond articular cartilage (for example, for regeneration of intervertebral discs and the meniscus). We hypothesize that evolving fabrication and printing approaches that enable researchers to functionally mimic cartilage architecture will facilitate advances in our endeavour to achieve true regeneration of articular cartilage.

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Competing interests
The authors declare no competing interests.

RHEUMATOID ARTHRITIS

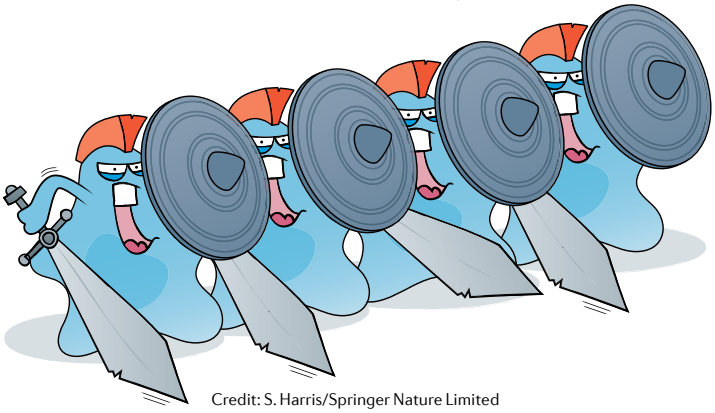
Synovial macrophages shield the joints

> CX_3CR1^+ synovial macrophages formed a distinct population that expressed several immune-related genes

Infiltrating macrophages are important mediators of inflammation in rheumatoid arthritis (RA), but surprisingly little is known about tissue-resident synovial macrophages and whether they have protective or destructive functions during disease. A new study has revealed insights into the previously mysterious lives of synovial macrophages in health and disease, including the identification of a subset of cells that create a protective shield around the joint.

"To visualize synovial macrophages and study their origin and spatiotemporal distribution during steady state and arthritis, we used various reporter mouse strains and fate-mapping approaches together with light sheet fluorescence microscopy," explains corresponding author Gerhard Krönke. "This approach provided us with information on the 3D distribution of distinct macrophage subsets within the joint."

Two main populations of synovial macrophages emerged from these studies on the basis of expression of the chemokine receptor CX_3CR1; CX_3CR1^+ lining macrophages and CX_3CR1^- interstitial macrophages. These macrophages were not derived from circulating monocytes. Instead, CX_3CR1^- interstitial macrophages seemed to be a self-renewing precursor to CX_3CR1^+ synovial macrophages.



Credit: S. Harris/Springer Nature Limited

In addition to following the fate of synovial macrophages using various imaging modalities, Krönke and colleagues also use single-cell RNA sequencing to examine the transcriptomes of these macrophage populations. CX_3CR1^+ synovial macrophages formed a distinct population that expressed several immune-related genes. By contrast, CX_3CR1^- interstitial macrophages could be further divided into several subsets, including actively proliferating cells and a terminally differentiated population characterized by the expression of the putative vascular remodelling hormone RELMα.

"The single and bulk RNA sequencing of the synovium is helpful to recognize the heterogeneity of the synovial macrophage population," states Harris Perlman, an expert on macrophages in arthritis who was not involved in this study. However, as with all genomics studies, the reproducibility of the raw and processed data will need to be verified with other studies."

By comparing single-cell RNA sequencing data on synovial cells from K/B × N mice with serum transfer-induced arthritis with similar data from the Accelerating Medicines Partnership on synovial cells from patients with RA, the researchers could tentatively match cell populations in mice and in humans. Two of the macrophage populations identified in humans matched tissue-resident synovial macrophages in mice, and two matched infiltrating monocyte-derived macrophages, although an exact match for CX_3CR1^+ lining macrophages could not be identified in the human data.

Imaging and fate-mapping studies during the development of either serum transfer-induced arthritis or collagen-induced arthritis in mice revealed further insights into the early stages of disease, including a change in the shape of CX_3CR1^+ lining macrophages in response to immune complexes that seems to precede tissue infiltration with immune cells.

"We observed that synovial lining macrophages form a distinct subset that organizes into membrane-like structures around the joint," says Krönke. "Interestingly, this specific macrophage subset shares many features with epithelial cells (including the expression of tight junction proteins) and provides an anti-inflammatory barrier around the joint that hinders immune cell trafficking in a steady state, but 'cracks' open during arthritis."

The researchers plan to further investigate synovial macrophage subsets in different forms of arthritis and to explore the possibility of targeting specific subsets therapeutically.

"The key to future studies will be to understand how each population of synovial macrophages contributes to pathology, and which ones might be the most important for targeted therapy; for example, does one population of synovial macrophages respond to biologics compared with another?" says Perlman.

Joanna Collison

ORIGINAL ARTICLE Culemann, S. et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature* https://doi.org/10.1038/s41586-019-1471-1 (2019)
RELATED ARTICLE Buckley, C. D. et al. Macrophages form a protective cellular barrier in joints. *Nature* https://doi.org/10.1038/d41586-019-02340-x (2019)

IN BRIEF

PAEDIATRIC RHEUMATOLOGY

Lung disease in sJIA has distinct features

Systemic juvenile idiopathic arthritis (sJIA)-associated lung disease is distinct from other inflammatory lung conditions, according to the results of new study. In patients with sJIA attending the Cincinnati Children's Hospital Medical Center, the presence of lung disease was associated with a young age of diagnosis, a history of macrophage activation syndrome and prior adverse reactions to cytokine-targeted biologic therapy. Lung disease in these patients shared some histopathological features with pulmonary alveolar proteinosis but had different immunological features, including increased IL-18.

ORIGINAL ARTICLE Schulert, G. S. et al. Systemic juvenile idiopathic arthritis-lung disease: characterization and risk factors. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41073 (2019)

SYSTEMIC SCLEROSIS

Protecting against myocardial disease in SSc

In a prospective observation study of 601 patients with systemic sclerosis (SSc), the use of vasodilators (including calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors and/or angiotension II receptor blockers) was associated with a reduced incidence of ventricular arrhythmias (hazard ratio (HR) 0.28, 95% CI 0.09–0.90). In the same multi-variant Cox regression analysis, low-dose acetylsalicylic acid (ASA) (≤325 mg daily) was associated with a reduced incidence of cardiac blocks and/or Q waves and/or pacemaker implantation (HR 0.46, 95% CI 0.24–0.87).

ORIGINAL ARTICLE Valentini, G. et al. Vasodilators and low-dose acetylsalicylic acid are associated with a lower incidence of distinct primary myocardial disease manifestations in systemic sclerosis: results of the DeSScipher inception cohort study. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2019-215486 (2019)

GENETICS

Two GWAS loci identified in IgG4-related disease

The first genome-wide association study (GWAS) of IgG4-related disease, involving 857 Japanese patients with IgG4-related disease and 2,082 healthy participants, has identified two susceptibility loci: *HLA-DRB1* and *FCGR2B*. The strongest disease association in *HLA-DRB1* corresponded to an amino acid residue in the peptide-binding groove of HLA-DRB1. The single nucleotide variant in *FCGR2B* (rs1340976) was associated with increased expression of *FCGR2B*, as well as with specific clinical features of IgG4-related disease (including the number of swollen organs and IgG4 concentration at diagnosis).

ORIGINAL ARTICLE Terao, C. et al. IgG4-related disease in the Japanese population: a genome-wide association study. *Lancet Rheumatol.* https://doi.org/10.1016/S2665-9913(19)30006-2 (2019)

THERAPY

Pregnancy outcomes in patients with JIA

Registry data from a long-term observation study of patients with juvenile idiopathic arthritis (JIA) suggest that DMARD exposure does not increase the risk of major adverse pregnancy outcomes in patients with JIA. Among the 152 pregnancies in 98 women with JIA and 39 pregnancies involving men with JIA as partners, the rates of miscarriage (13.1%) and major congenital anomaly (3.6%) were similar to the expected background rates. Half of the pregnancies were unplanned and occurred during treatment with DMARDs. Elective abortions were also common in DMARD-exposed pregnancies.

ORIGINAL ARTICLE Drechsel, P. et al. Pregnancy outcomes in DMARD-exposed patients with juvenile idiopathic arthritis—results from a JIA biologic registry. *Rheumatology.* https://doi.org/10.1093/rheumatology/kez309 (2019)

CLINICAL GUIDELINES

Vaccination guidance updated

Prevention of infection is important in the management of autoimmune inflammatory rheumatic diseases (AIIRD), but uptake of vaccinations is suboptimal in patients with AIIRD worldwide. In light of new data on the prevalence and incidence of vaccine-preventable infections in adults with AIIRD, as well as on the efficacy, immunogenicity and safety of available vaccines, EULAR has issued updated recommendations for vaccinations in these patients.

The 2019 update comprises six overarching principles and nine recommendations, formulated by an international group of experts and based on a comprehensive systematic literature review. "Since the first version of EULAR recommendations on vaccination of adult patients with AIIRD was published in 2011, there has been a large expansion in the amount of available evidence on this topic, necessitating an update," says lead author Victoria Furer.

Notably, the EULAR task force used clear outcome measures of vaccination when evaluating this evidence. "In the AIIRD population, the data on the clinical efficacy of vaccination is limited," explains Furer. "Thus, 'immunogenicity' of vaccination was used as a surrogate marker of efficacy, when appropriate. The strength of recommendations was based on the level of the data. For example, in case of lack of a direct correlation between the immunogenicity outcomes and the level of protection, the strength of recommendation was downgraded."

The overarching principles stress the need for regular assessment, patient education and shared decision-making. "We hope that this recommendation will improve the implementation of the vaccination programme," notes Furer. The principles also state that vaccines should be administered during quiescent disease and before planned immunosuppressive therapy, and that non-live vaccines can be given to patients being treated with glucocorticoids and/or DMARDs. Live-attenuated vaccines should be avoided during immunosuppression but, in a modification of the 2011 recommendations, MMR and herpes zoster vaccines can be considered with caution.

The core set of recommendations concerning influenza, pneumococcal, tetanus toxoid, hepatitis A, hepatitis B and HPV vaccinations remained essentially unchanged from the 2011 recommendations, with some minor modifications. Several of the 2011 recommendations were omitted from the 2019 update; two, concerning BCG vaccination and vaccination of hyposplenic or asplenic patients with AIIRD, had become irrelevant to clinical practice and one, concerning travelling patients, was deemed non-specific. New recommendations were added to encourage the vaccination of immunocompetent members of the households of patients with AIIRD, to avoid vaccination with live-attenuated vaccines for the first 6 months of life in newborns exposed to biologic drugs during the late stages of pregnancy and to avoid vaccination against yellow fever in patients with AIIRD during immunosuppression.

"The implementation of the present recommendations will help in prevention of infections in the susceptible population of patients with AIIRD," Furer contends. "In particular, dissemination of the main principles of these recommendations among the health professionals, including primary care teams, treating patients with AIIRD is of great importance. Thus, a number of educational projects for the medical community to increase the awareness of vaccination and compliance with the recommendations are underway."

Sarah Onuora

ORIGINAL ARTICLE Furer, V. et al. 2019 update of EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2019-215882 (2019)

SYSTEMIC SCLEROSIS

DDP4 inhibition reduces fibrosis

Tissue fibrosis and persistent activation of fibroblasts are hallmark features of systemic sclerosis (SSc). Findings from a new study highlight the serine protease dipeptidyl-peptidase-4 (DPP4, also known as CD26) as not only a marker of activated fibroblasts in SSc, but also as a potential therapeutic target for treating fibrosis in SSc.

Inhibitors of DPP4 (such as sitagliptin) are already approved and widely used for the treatment of diabetes mellitus owing to the ability of DPP4 to target and inhibit incretin hormones. However, DPP4 also has a broad range of other substrates, including chemokines, and can also function independently of its enzymatic activity, bestowing DPP4 with a broad range of functions.

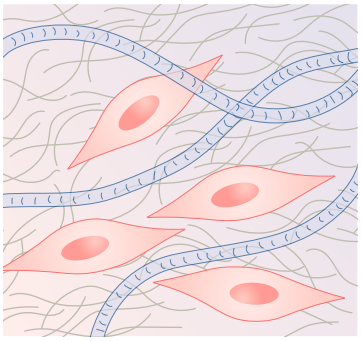
In these latest findings, the investigators found that the expression of DPP4 and the number of DPP4-expressing fibroblasts is upregulated in the skin of patients with SSc compared with the skin of healthy individuals.

Incubation of human dermal fibroblasts with recombinant transforming growth factor-β (TGFβ), a key profibrotic cytokine in SSc, induced the expression and enzymatic activity of DDP4 in vitro. This upregulation was dependent on non-canonical TGFβ signalling via the kinase ERK.

In fibroblasts from either mice or humans, the expression of DDP4 was associated with increased expression of myofibroblast markers and type I collagens as well as with increased responsiveness of the fibroblasts to stimulation with TGFβ. Notably, treatment of fibroblasts from patients with SSc with sitagliptin inhibited TGFβ-induced fibroblast-to-myofibroblast transition and release of type I collagens.

Loss of DPP4 activity through gene knockout or treatment with sitagliptin ameliorated disease in mice with bleomycin-induced dermal or pulmonary fibrosis. Importantly, treatment could also induce regression of pre-established fibrosis

> “Loss of DPP4 activity through gene knockout or treatment with sitagliptin ameliorated disease”



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in mice with bleomycin-induced fibrosis as well as in mice with graft-versus-host disease.

The authors speculate that a subpopulation of DPP4-positive fibroblasts promote tissue fibrosis in SSc, and they plan to use lineage tracing experiments to confirm this theory. They also plan to use additional experimental models to further confirm the antifibrotic effects of this enzyme, with the long-term aim of a clinical trial in SSc.

Jessica McHugh

ORIGINAL ARTICLE Soare, A. et al. Dipeptidyl-peptidase-4 as a marker of activated fibroblasts and a potential target for the treatment of fibrosis in systemic sclerosis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41058 (2019)

AUTOIMMUNITY

Placental growth factor links angiogenesis and autoimmunity

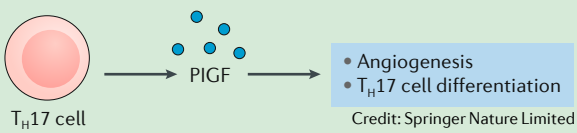
Increased angiogenesis and immune cell infiltration go hand-in-hand at sites of inflammation in autoimmune diseases, such as rheumatoid arthritis (RA). The results of a new study suggest that placental growth factor (PlGF), a vascular endothelial growth factor homologue, could help to mediate both processes by stimulating angiogenesis and T helper 17 (T_H17) cell differentiation.

“Although angiogenesis and T cell infiltration are tightly interwoven processes in both health and disease, it has not been clear whether angiogenic factors affect T helper cell differentiation or whether a specific T helper cell subset directly contributes to pathologic angiogenesis in autoimmune diseases,” explains corresponding author Wan-Uk Kim.

> “PlGF was secreted specifically by activated T_H17 cells”

To address this uncertainty, Kim and colleagues investigated the potential role of PlGF in crosstalk between endothelial cells and T cells. In vitro, PlGF was secreted specifically by activated T_H17 cells, and T cell-secreted PlGF could stimulate neovascularization both in vitro and in vivo.

The addition of PlGF-conditioned media or recombinant PlGF to $CD4^+$ T cells caused upregulation of the T_H17 cell-specific transcription factor RORγ. PlGF-mediated RORγ upregulation required the phosphorylation of signal transducer and activator 3, similar to IL-6-mediated signalling during classical T_H17 cell differentiation. Interestingly, PlGF could stimulate T_H17 cell differentiation in the absence of IL-6.



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Placing these findings in the context of autoimmune disease, overexpression of PlGF in T cells exacerbated disease in mice with collagen-induced arthritis. PlGF concentrations also correlated with IL-17 concentrations in synovial fluid from patients with RA.

“Our findings provide novel insights into PlGF-mediated links between angiogenesis, T_H17 cell development and autoimmunity, indicating that PlGF inhibitors might be able to control autoimmune and inflammatory diseases via the dual inhibition of angiogenesis and T_H17 cell generation,” says Kim.

The researchers are currently looking to develop such PlGF inhibitors for use in diseases with T_H17 cell involvement, such as RA.

Joanna Collison

ORIGINAL ARTICLE Yoo, S.-A. et al. Placental growth factor regulates the generation of T_H17 cells to link angiogenesis with autoimmunity. *Nat. Immunol.* https://doi.org/10.1038/s41590-019-0456-4 (2019)

RHEUMATOID ARTHRITIS

CCL21–CCR7 axis in RA: linking inflammation and bone erosion

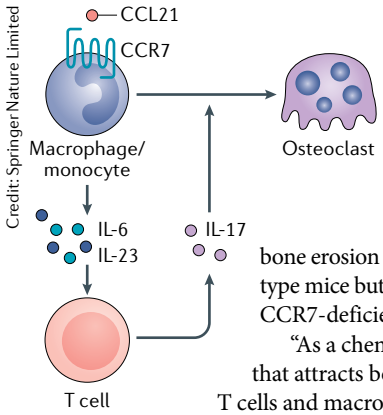
Rheumatoid arthritis (RA) is characterized by joint inflammation and bone erosion mediated by excessive production of pro-inflammatory mediators. A new study highlights the importance of the chemokine CCL21 and cross-talk between macrophages and T cells in this destructive process.

In characterizing the pathogenic function of CCL21, the researchers found that the expression of its receptor CCR7 on monocytes was higher in patients with RA than in healthy individuals, and correlated with the patient's 28-joint disease activity score (DAS28).

In vitro, monocyte chemotaxis, induced by treatment with synovial fluid from patients with RA, was reduced by neutralization of CCL21 or CCR7. Furthermore, treatment with CCL21 promoted the chemotactic activity of monocytes in a CCR7-dependent manner and upregulated the transcription of IL-6 and IL-23 (cytokines involved in T helper 17 (T_H17) cell differentiation).

Given the important function of T_H17 cells and IL-17 production in RA, the researchers assessed the effects of CCL21 on T cells in vitro. CCL21 treatment promoted IL-17 secretion by peripheral blood mononuclear cells (PBMCs), but not by T cells alone. Furthermore, CCL21 treatment promoted osteoclastogenesis by PBMCs, which was inhibited by antibodies against IL-6 receptor (IL-6R), IL-23 or IL-17.

These findings together suggest that CCL21 induces polarization of T_H17 cells in a myeloid cell-dependent manner, which in turn stimulates osteoclast formation and bone erosion. Notably, adenovirus-mediated expression of CCL21 (via intra-articular injection) promoted joint inflammation and bone erosion in wild-type mice but not in CCR7-deficient mice.



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> CCL21 induces polarization of T_H17 cells in a myeloid cell-dependent manner

"As a chemokine that attracts both T cells and macrophages, promotes myeloid and T cell pathogenic activity and is an RA susceptibility gene, CCL21 is ideally positioned to instigate disease and thus makes for a promising novel therapeutic target," explains Katrien Van Raemdonck, first author of the study. "Further research will have to verify whether CCL21–CCR7 activity can be inhibited efficiently and safely for therapeutic purposes."

Jessica McHugh

ORIGINAL ARTICLE Van Raemdonck, K. et al. CCL21/CCR7 signaling in macrophages promotes joint inflammation and Th17-mediated osteoclast formation in rheumatoid arthritis. *Cell. Mol. Life Sci.* https://doi.org/10.1007/s00018-019-03235-w (2019)

IMMUNOMETABOLISM

Faulty mitochondrial DNA repair promotes inflammation in RA

In rheumatoid arthritis (RA), T cells age prematurely and have defects in DNA repair mechanisms and a distinct metabolic signature. New research suggests that these characteristics might be linked to each other, and to inflammation, via the mitochondria.

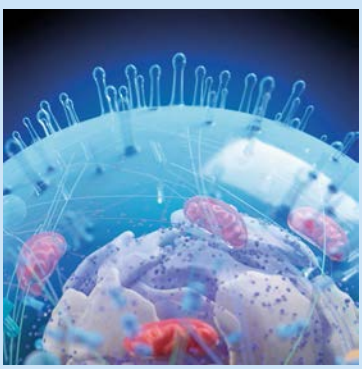
Previous studies had identified low expression of the DNA repair nuclease MRE11A as being linked to the prematurely aged phenotype of RA T cells. In the new study, inducing low MRE11A expression in otherwise healthy T cells produced a phenotype similar to that of T cells from patients with RA, in which mitochondrial function was impaired. Interestingly, the low expression of MRE11A in RA T cells extended to the mitochondria, where it is also present, and was linked to DNA damage and leakage.

"We had to leave our comfort zone and transition from the nucleus to the mitochondria to study how MRE11A contributes to mitochondrial failure in RA T cells," explains corresponding author Cornelia Weyand. "We developed techniques to study how mitochondrial DNA (mtDNA) leaks into the cytoplasm and how leaked mtDNA is recognized by DNA sensors."

> *MRE11A* knockdown in T cells resulted in increased caspase-1 activation and synovial tissue inflammation

The researchers found that leaked mtDNA triggered the inflammasome in T cells with low MRE11A expression, causing the activation of caspase-1, the release of IL-1β and pyroptotic cell death. In a humanized model of RA in which human synovial tissue was engrafted into NSG mice, *MRE11A* knockdown in T cells resulted in increased caspase-1 activation and synovial tissue inflammation. By contrast, *MRE11A* overexpression in T cells reduced synovial inflammation in this model.

"Implicating mtDNA repair in the tissue inflammatory response in RA comes as quite a surprise," says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Weyand. "We next want to explore the therapeutic implications of mitochondrial infidelity in RA. Can we prevent the leakage of mtDNA into the cytoplasm? Can we prevent pyroptotic T cell death? Can we repair the damaged DNA within the mitochondria, and will that rescue mitochondrial function?" Future studies will hopefully ascertain whether mtDNA repair is indeed a feasible therapeutic target for RA.

Joanna Collison

ORIGINAL ARTICLE Li, Y. et al. The DNA repair nuclease MRE11A functions as a mitochondrial protector and prevents T cell pyroptosis and tissue inflammation. *Cell Metab.* https://doi.org/10.1016/j.cmet.2019.06.016 (2019)

THERAPY

Could a methotrexate blood assay improve adherence?

Maxime Dougados

In patients with chronic inflammatory rheumatic diseases, non-adherence to methotrexate therapy could lead to lower drug efficacy, unnecessary adjustments of medication, and avoidable health-care costs. Use of a novel blood assay to measure methotrexate could help prevent or reduce non-adherence in clinical practice.

Refers to Bluett, J. et al. Development and validation of a methotrexate adherence assay. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2019-215446 (2019).

In the absence of contraindications, methotrexate (MTX) is the recommended first-line DMARD for Rheumatoid Arthritis (RA)[1]. Optimal MTX therapy use has several benefits, including the control of disease, a reduced need for more expensive biologic treatments, and improvements in health outcomes[2]. Unfortunately, not all patients respond to MTX, which might be partially explained by non-adherence. In a new study, James Bluett and colleagues propose a new assay that permits rapid and easy measurements of MTX plasma concentrations in patients with chronic inflammatory rheumatic diseases receiving weekly low-dose MTX[3]. Could this assay be used in the future as part of a biofeedback tool for improving adherence behaviour of patients?

> “non-adherence might lead to complications and unnecessary treatment switches”

As a first step, the researchers developed a high-performance liquid chromatography–selected reaction monitoring–mass spectrometry (HPLC-SRM-MS)-based assay for the detection of MTX and its major metabolite 7-hydroxy-MTX[3]. Using a pharmacokinetic model, they determined the adherence cut-offs required for the correct detection of adherence according to the dose of MTX ingested. Thereafter, Bluett et al. validated this assay in a group of patients participating in a 1 year prospective multicentre observational study designed to identify predictors of response to MTX in patients with RA. This assay was able to identify patients who were adherent to MTX, with a sensitivity of 95%.

This very interesting study raises several questions concerning MTX adherence in daily clinical practice (BOX 1). First, how common is and what is the impact of non-adherence to MTX? Although physicians tend to overestimate how well patients take their medication as prescribed[2], long-term adherence to MTX is only moderate and varies across studies, with reported rates of adherence ranging from 40% (underuse) to 107% (overuse)[4–6]. Non-adherence to DMARDs can be detrimental, leading to lower drug efficacy and potential cost increases[7]. Furthermore, non-adherence might lead to complications and unnecessary treatment switches[8].

Another question to consider is whether some patients are at a higher risk than others of non-adherence and, if so, whether we can identify such patients before commencing treatment. Multiple factors that influence non-adherence have been reported across studies and across diseases, with different results, making the findings difficult to interpret[2]. For this reason, current recommendations propose that all patients are screened for non-adherence[2]. Moreover, factors involved in ‘unintentional’ non-adherence (for example, patients simply forgetting to take their medication) and ‘intentional’ non-adherence (for example, non-adherence because of a patient’s beliefs and/or fears linked to their medication) are often intertwined. Patients often cite unintentional causes of non-adherence such as having forgotten to take the drug, but health professionals should be aware that this excuse might hide other reasons for non-adherence.

The most important question to consider is whether non-adherence to drugs is preventable. Results from studies and in particular from randomized controlled studies conducted in chronic disorders, including in disorders other than rheumatic diseases, suggest that the best way to reduce drug non-adherence is to prevent it[8]. To optimize drug adherence, it is strongly recommended that patients are involved in decisions surrounding their own healthcare (shared decision-making) and that patient information and education accompany any anti-rheumatic treatment[2].

But if a patient is non-adherent to their ongoing medication, how can adherence be improved? It could be argued that any assessment of treatment adherence permits a discussion between the health professional and the patient. This discussion should remind the patient of the importance of optimal drug adherence and, therefore, guide towards helping improve drug adherence. As well as patient education, studies have assessed the benefits of other interventions such as behavioural interventions, cognitive behavioural interventions or motivational interviewing, with conflicting results[5,9].These interventions necessitate access to an expert in these approaches, which is rare and probably the reason why these interventions are seldom proposed in daily practice.

Box 1 | **Methotrexate adherence**

- How common is and what is the impact of non-adherence to methotrexate in clinical practice?
- Are some patients at higher risk than others of non-adherence and, if so, can we identify such patients?
- Is non-adherence to methotrexate preventable?
- How can we improve methotrexate adherence during ongoing treatment?
- How can methotrexate adherence be assessed in clinical practice? And when is the best time to evaluate adherence?

> this approach might lead to changes in the procedure for managing MTX therapy in daily practice

So how can MTX adherence be assessed in clinical practice? The assessment of drug adherence in clinical trials can be on the basis of very sophisticated techniques such as the use of pill-counting devices. The current recommendation is that during any single outpatient visit, at least one question should be posed to the patient to evaluate adherence[2]; however, this simple approach has limitations, particular with regard to its validity, which could probably explain why, in daily practice, drug adherence is rarely assessed by the physician, at least in the context of MTX intake.

In the field of rheumatology, an example of a blood test frequently used in daily practice to assess drug adherence already exists: the evaluation of hydroxychloroquine in patients with systemic lupus erythematosus (SLE)[10]. Measurements of hydroxychloroquine blood concentrations and discussions of these results with the patient (especially if the drug concentrations are low) have been shown to improve adherence to hydroxychloroquine[10]. By contrast, an alternative method — simple text messaging reminders without hydroxychloroquine measurements — was not sufficient to improve adherence of these patients.

The use of a blood test for assessing concentrations of a drug can be considered as both a preventive strategy (by informing the patients of this blood test before treatment) and also a curative strategy as the results of the blood test can be used as a starting point for the discussion of drug adherence between the patient and the health professionals.

Obviously, the proposed blood test for MTX has some limitations[3]; in particular, the assay can only detect whether the drug was taken, and taken at the correct dose, within the preceding 6 days, which does not reflect long-term adherence. The assay would be unable to detect, for example, non-adherence during the majority of treatment except in the few days preceding the patients' appointments. Hence, the best time to use this assay is questionable. It could be argued that a systematic assessment (for example, every trimester or semester) might be useless in patients with a disease perfectly controlled by their current treatment. By contrast, this assessment might be of greater benefit for patients for whom a switch or an add-on strategy is being considered because of an inadequate response and/or a loss of response to MTX. These strategies (switching or adding on therapies) might be more toxic and/or more costly (in particular, if involving the initiation of biologic drugs) compared with improving or preventing poor MTX adherence.

Hence, despite the limitations discussed above, this plasma MTX assay deserves further evaluation. Further clinical studies should investigate whether measurement of MTX adherence using the assay can improve MTX adherence. Ultimately, this approach might lead to changes in the procedure for managing MTX therapy in daily practice, and could have a beneficial effect on patient outcomes and decrease the need to initiate other more expensive and toxic drugs such as biologic drugs in many patients.

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Competing interests
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GOUT

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The 2018 EULAR guideline recommends a three-step approach for the diagnosis of gout[2]. The first step is to demonstrate the presence of monosodium urate (MSU) crystals in synovial fluid or tophus aspirates, using polarised light microscopy, in every person with suspected gout. The second step, when MSU crystal demonstration is not feasible, is to make a clinical diagnosis on the basis of clinical features that are suggestive of and associated with

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gout and the presence of hyperuricaemia. The third step is to use imaging, particularly ultrasonography or dual-energy CT (DECT), to search for evidence of MSU crystal deposition when a clinical diagnosis of gout is uncertain and crystal identification is not possible. EULAR put forth eight evidence-based consensus statements regarding the diagnosis of gout that provide more details regarding these three steps. Notably, there was consensus that a diagnosis of gout should not be based on the presence of hyperuricaemia alone[2].

So, what is the purpose of these updated 2018 EULAR recommendations for gout diagnosis? The authors cite new data related to the use of DECT, ultrasonography and other imaging modalities in the diagnosis of gout, as well as a diagnostic algorithm proposed in 2010 (REF.[3]), that have emerged since the publication of the last EULAR recommendations for gout diagnosis in 2006 (REF.[4]). A major difference between the 2006 and the 2018 EULAR gout diagnosis guidelines is that the lack of imaging data in 2006 led to a recommendation that its role in gout diagnosis be investigated[4], whereas the 2018 guideline includes a recommendation to use imaging for gout diagnosis in cases of diagnostic uncertainty or when MSU crystal documentation is not possible[2]. Ultrasonography, which reveals the 'double-contour' sign, tophi and the 'snow-storm' appearance as signs for gout, has a specificity for gout of 84%[2], which is good, but not perfect. DECT can characterize, quantify and map MSU crystal deposition and detect deep-seated structures and/or regions and has > 90% specificity and 78–91% sensitivity for gout[2]. However, both DECT and ultrasonography have important limitations, such as their lower sensitivity in early disease and in gout without tophi, which together account for a substantial proportion of gout cases[5]. Sometimes artefacts on DECT can resemble MSU, although they can be distinguished using low-voltage and high-voltage DECT images, techniques such as Z-effective maps, or even other imaging modalities, as shown in FIG. 1.

The American College of Physicians (ACP) issued a guideline for the diagnosis of gout in 2017 (REF.[6]) that contrasts with the 2018 EULAR recommendations. The ACP guideline recommends that clinicians assess synovial fluid for MSU crystals "when clinical judgment indicates that diagnostic testing is necessary in patients with possible acute gout" (presented as a weak recommendation based on low-quality evidence)[6]. This recommendation contrasts with the EULAR gout diagnosis guideline that synovial fluid or tophus aspiration should be performed in every case of suspected gout[2], so that the presence of MSU crystals — the central pathological feature of gout — can be established. The ACP guideline acknowledged that synovial fluid aspiration and analysis is considered the reference standard (that is, the gold standard) for the diagnosis of gout, but noted that this procedure could be difficult to perform in primary care. In contrast to the moderate-quality evidence rating for clinical algorithms, the ACP rated the evidence for DECT (sensitivity 85–100%, specificity 83–92%) and ultrasonography (sensitivity 74%, specificity 88%) for the diagnosis of gout as low-quality[6]. The wide gap between these diagnostic recommendations from two professional organizations creates a lot of confusion for a practicing physician.

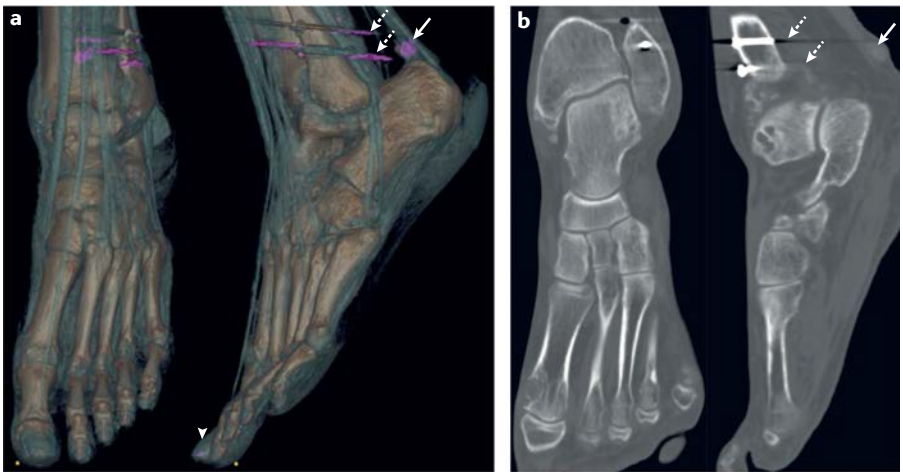

Fig. 1 | **True-positive, false-positive and false-negative imaging findings for MSU deposition in a 68-year-old man with tophaceous and erosive gout.** Dual-energy CT (DECT) (**a**) and coronal and sagittal conventional CT (**b**) images show monosodium urate (MSU) deposition in pink. The subcutaneous tophus (arrow) corresponds to true MSU (true-positive). Typical DECT artefacts also appear pink (false-positives), including artefacts related to metal screws (dashed arrows) and pseudo-MSU deposition (arrowhead). Notably, DECT failed to identify subtle MSU deposition within a typical gouty bone erosion in the first metatarsal bone, which was confirmed through joint and tophus aspiration (false-negative). Images in parts **a** and **b** courtesy of F. Becce, Lausanne University Hospital, Switzerland.

According to the ACP guideline[6], synovial fluid aspiration should be performed only in certain clinical circumstances: the joint aspiration can be done "without substantial patient discomfort by an experienced clinician who can minimize the risk of infection"; a polarising microscope and a trained operator are available; and the clinical situation is ambiguous and a probability of infection exists. These clinical circumstances imply that the use of this procedure would be limited to a small minority of people with gout, which is amazingly consistent with the low rates of joint aspiration-assisted MSU crystal-proven gout in the USA[7]. With more research and data, emerging techniques, such as the point-of-care Raman spectroscopy-based device for quick, easy and automated detection of MSU, might complement or replace polarised light microscopy[8].

Rather than limiting the use of the gold-standard test for gout[7], a better approach might be for primary care physicians or family practitioners to gain more experience in performing aspiration of joints such as the knee, ankle, great toe, wrist etc., which are commonly affected by gout. Acute inflammation of the first metatarsophalangeal joint does not equate to gout every time; it can also be caused by other conditions. Calcium pyrophosphate (CPP) deposition disease (CPDD; formerly called CPPD) and erosive osteoarthritis, which might both be just as prevalent as gout, can (and often do) have a presentation similar to gout. Septic arthritis is also a concern for an acutely swollen big toe, although rare. Moreover, owing to the obesity epidemic, hyperuricaemia without gout is common[2], and can coexist with CPDD or OA. Similarly, acute inflammation of ankle, knee or midfoot joints cannot be presumed to be gout automatically. Assuming an acutely swollen joint is caused by gout, and not performing aspiration to confirm the diagnosis of gout[7], is a missed opportunity in urgent, emergency and primary care settings. This practice must change. The new EULAR recommendations provide clear guidance for this in a simple, three-step approach.

Can new imaging techniques have a role in gout diagnosis? DECT and ultrasonography are already being used in clinical diagnosis of gout. Emerging imaging techniques, such as

multi-energy photon-counting CT[9] and spectral photon-counting radiography[10], could have a role in the diagnosis of gout in the future, by offering better contrast and spatial resolution (~100 μm and ~50 μm, respectively) than the currently available techniques. Multi-energy photon-counting CT can better distinguish MSU from CPP crystal deposits than DECT[9]. Spectral photon-counting radiography[10] should avoid the need for DECT in some cases of suspected gout. The use of these techniques will complement the imaging techniques currently available, and when used in combination with them will help to better distinguish MSU from other crystal arthritis conditions in the future. Studies need to be performed to establish the accuracy of these emerging techniques, and if their accuracy (that is, overall sensitivity and specificity combined) is high, these techniques have the potential to provide an accurate non-invasive quantification and mapping of the MSU crystal deposition in the entire body. In particular, these techniques might assist in the assessment of MSU deposition in asymptomatic disease, either in the pre-gout stage or for people with established gout and symptomatic control of flares.

Future advances in imaging techniques will further improve our understanding of gout, but are unlikely to replace synovial fluid analysis as the gold standard for gout diagnosis. Physician inertia results in reliance on clinical judgement rather than using the gold-standard test — that is, joint fluid aspiration, a procedure that can directly document the disease pathology and that is safe, practical and low-cost. This inertia is at least partly related to lack of experience in aspirating common joints (such as the knee, ankle and toe) and perception of the time needed to perform joint aspiration. A good health care provider must do what is best for the patient, not what is easiest. The new 2018 EULAR gout diagnosis guideline, if followed, will not only lead to a more accurate and earlier diagnosis of gout, but also avoid missed and delayed diagnosis.

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Competing interests

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Mechanisms of lung disease development in rheumatoid arthritis

Dan Wang[1], *Jie Zhang*[2], *Jessica Lau*[3], *Shaohua Wang*[3], *Veena Taneja*[4], *Eric L. Matteson*[5] *and Robert Vassallo*[3,6]*

Abstract | Rheumatoid arthritis (RA) is a chronic autoimmune disorder that causes joint inflammation and damage. Extra-articular manifestations occur in many patients and can include lung involvement in the form of airway or parenchymal inflammation and fibrosis. Although the pathophysiology of articular RA has been extensively investigated, the mechanisms causing airway and parenchymal lung disease are not well defined. Infections, cigarette-smoking, mucosal dysbiosis, host genetics and premature senescence are all potentially important contributors to the development of lung disease in patients with RA. RA-associated lung disease (which can predate the onset of articular disease by many years) probably originates from chronic airway and alveolar epithelial injury that occurs in an individual with a genetic background that permits the development of autoimmunity, leading to chronic inflammation and subsequent airway and lung parenchymal remodelling and fibrosis. Further investigations into the specific mechanisms by which lung disease develops in RA will be crucial for the development of effective therapies. Identifying mechanisms by which environmental and host factors cooperate in the induction of autoimmunity in the lung might also help to establish the order of early events in RA.

Rheumatoid arthritis (RA) is a systemic inflammatory disease that is characterized by joint swelling, pain and morning stiffness[1]. Over the course of the disease, extra-articular manifestations occur in up to half of patients with RA and can affect the skin, eyes, heart, kidneys, nervous system, gastrointestinal tract and lungs[2], potentially making the term 'rheumatoid disease' a more fitting description of the multi-systemic nature of RA. These extra-articular manifestations influence the natural course of disease and substantially affect RA-associated morbidity and mortality[2].

Host factors and environmental factors are both involved in the pathogenesis of RA (FIG. 1). Substantial data support a model in which RA develops after a pre-clinical period (the so-called pre-RA phase) that precedes the onset of symptoms by months to years (and potentially decades)[3]. This preclinical period is characterized by the emergence of autoantibodies that are detectable in the circulation and the lung, as well as evidence of systemic inflammation (increased concentrations of pro-inflammatory cytokines and chemokines) occurring in the absence of articular symptoms[4]. Lung disease in RA can either precede the onset of articular symptoms or become clinically evident after joint manifestations occur. In this Review, we discuss mechanisms by which smoking, host genetics and other factors can induce lung disease in patients with RA. We propose mechanisms by which dysregulation of immune responses to self-antigens in the lung might be relevant to the generation of systemic autoimmunity in RA, and suggest important avenues for future investigations into the cause of lung complications in RA.

The lung in the development of RA

Epidemiological, clinical and molecular studies in seropositive individuals at risk of developing RA, as well as in individuals with untreated early-stage RA, support a role for mucosal sites as the region of origin for RA-related autoimmunity. This 'mucosal origins' hypothesis suggests that the initiating events that precede symptomatic RA might originate at one or more mucosal sites (principally the oral, airway or gut mucosa)[3]. Although several lines of evidence support the mucosal origins hypothesis (reviewed elsewhere[3]), many questions remain regarding the mechanisms involved. In particular, how is it that local dysregulated immunity in the lung causes a systemic autoimmune process resulting in arthritis and other systemic manifestations?

A cardinal feature of seropositive RA is the generation of anti-citrullinated protein antibodies (ACPAs). These autoantibodies can be generated at synovial sites and at extra-articular sites, including the lung[5], and can be found years before the occurrence of articular symptoms[6]. Of particular relevance to a mucosal origins

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Key points

- Rheumatoid arthritis (RA) is a systemic autoimmune disease that can present with a variety of lung manifestations including airway disease and interstitial lung disease.
- Seropositive RA develops following an asymptomatic pre-RA phase characterized by the emergence of autoantibodies and systemic immune activation that might be initiated at mucosal surfaces such as the lung.
- Cigarette smoking, host genetic factors, dysbiosis in the oral cavity and airways and senescence are all potentially important in the pathogenesis of lung disease in RA.
- Identifying specific mechanisms that permit the breakdown of tolerance and generation of disease in the lung are important for the development of therapies that address lung complications in RA.
- Screening individuals with RA at risk of lung complications is now feasible and should be the focus of future studies.

Bronchoalveolar lavage (BAL). A medical procedure during which a bronchoscope is guided from the oral cavity or nose into the lungs for the purpose of instilling sterile fluid into a region of the lung and then aspirating back the fluid for examination.

Bronchiectasis A chronic disorder of the airways characterized by bronchial wall thickening and impaired mucous clearance, often associated with secondary colonization with various types of bacteria and other microorganisms.

Interstitial lung disease (ILD). A large collection of diseases that affect the interstitial spaces within the lungs.

Rheumatoid nodules Well-demarcated, subcutaneous lumps that vary in size and usually occur adjacent to joints on extensor surfaces, such as the elbow; they can also occur internally, such as in the sclera of the eyes, lungs or vocal cords.

hypothesis is the discovery of IgA ACPAs in sputum samples from individuals at risk of developing RA[7], as IgA antibodies are important in regulating mucosal defences. In this study[7], at-risk individuals included those with a first-degree relative with RA (defined by ACR criteria)[8] or individuals identified through community health fair screenings as being seropositive for ACPAs. RA-related autoantibodies were identified in the serum and induced sputum of at-risk seropositive individuals and patients with early-stage RA[7]. Notably, autoantibodies could also be detected in the sputum, but not in the serum, in seronegative individuals at risk of developing RA[7], suggesting that the lung could be the primary site of ACPA generation[9].

Further insight into the role of the lung as the primary site of RA initiation was provided by an investigation into immune activation in the lungs of patients with early-stage RA without lung disease. In this study[5], ACPA-positive individuals had higher concentrations of ACPAs in bronchoalveolar lavage (BAL) samples than in serum samples, and also had substantial evidence of immune activation in bronchial tissue. In the context of heightened bronchial immune cell activation, molecular mimicry to environmental antigens — primarily infectious pathogens — is probably an important process by which mucosal ACPAs are generated. In support of this concept, patients with bronchiectasis without RA have increased circulating serum concentrations of ACPAs compared with healthy individuals, although these autoantibodies are usually not specific for the citrulline element in the protein[10]. These findings suggest that a chronic purulent airway disease is sufficient for the generation of ACPAs, even in the absence of systemic autoimmunity[10].

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In general, the airway mucosa is possibly of greater relevance in the initiation of RA-related autoimmunity than other mucosal sites such as the gastrointestinal or genitourinary tract; it is within the airway mucosa that environmental exposures that predispose to both RA and lung disease (such as cigarette smoke[9,11–14] and silica dust[15]) interact with host factors and the local microbiome, resulting in local injury and inflammation that subsequently promote autoimmunity[16].

Lung disease in RA

Almost every lung compartment can be affected by RA-associated lung disease, including the large and small airways, pleura, pulmonary vessels and the interstitial compartment, and it is estimated that up to 60% of patients with RA will develop lung manifestations during the course of the disease[17,18]. Clinically, lung involvement can manifest as different patterns of interstitial lung disease (ILD), rheumatoid nodules, pulmonary hypertension, pleural disease, upper airway disease (such as cricoarytenoiditis) or lower airway disease (such as bronchiectasis, constrictive bronchiolitis and follicular bronchiolitis)[19–21] (FIG. 2). RA-associated lung disease usually manifests after the onset of articular symptoms; however, pulmonary manifestations can precede the onset of articular RA and, in some instances, ILD, bronchiectasis or obliterative bronchiolitis can predate the onset of arthritis by many years[22]. Different terms have been used to describe instances when patients develop a fibrotic ILD with positive ACPA but without other clinical features of RA. 'Lung-limited RA', 'autoimmune featured ILD' and 'interstitial pneumonia with autoimmune features' all refer to this entity. Current expert opinion favours interstitial pneumonia with autoimmune features as the preferred term[23].

Epidemiology and classification

The reported prevalence of lung manifestations in patients with RA varies depending on the sensitivity of the methods used (TABLE 1). For example, symptomatic ILD reportedly occurs in 5–17% of patients with RA[22,24,25], and symptomatic large or small airway involvement associated with cough or shortness of breath has been reported in up to 30% of patients with RA[18,26]. However, the prevalence of radiographically evident lung manifestations in patients with RA is higher than that of symptomatic disease, with radiographically evident ILD occurring in ~30% of patients with RA, and airway disorders (including both large and small airway diseases) occurring in 60% of patients[27,28] (TABLE 1). Symptomatic RA-associated pleural effusion is rarer than ILD or airway diseases, with an overall incidence of 3–5% and an annual incidence rate of <2% for men and 1% for women[29,30]. Furthermore, in a high-resolution CT (HRCT) study of 77 patients with RA, pulmonary nodules (some of which might be cavitary and raise clinical concern for cavitary malignancy or infection) were found in 22% of patients[31,32].

Overall, RA-associated lung disease represents a spectrum of phenotypic manifestations that could be further classified into endotypic categories on the basis of the pathophysiological mechanisms involved.

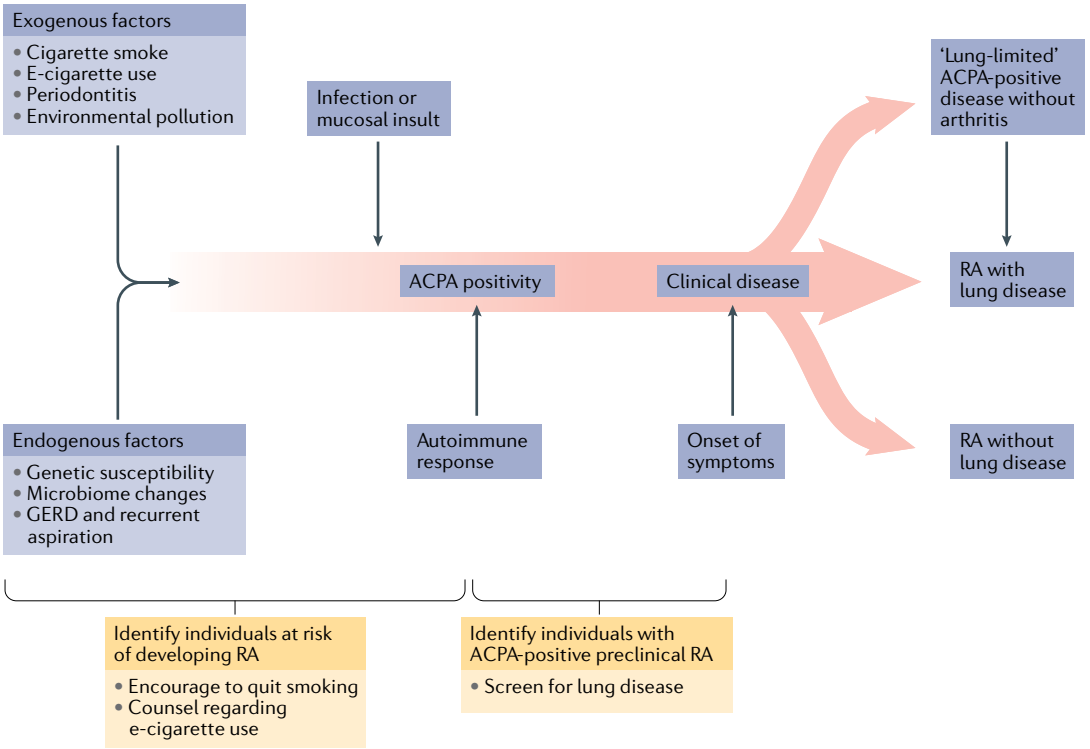

Fig. 1 | **The natural history of RA-associated lung disease.** A combination of endogenous host factors and potentially modifiable exogenous factors predispose individuals to the development of rheumatoid arthritis (RA) and RA-associated lung disease. An acute infection or tissue insult at a mucosal surface (such as the airways) is thought to result in the development of autoimmunity and the production of anti-citrullinated protein antibodies (ACPAs) in genetically predisposed individuals. Following this initial event, individuals can be asymptomatic or minimally symptomatic for many years. ACPA-positive individuals with preclinical RA can develop either airway disease or interstitial lung disease (ILD) with no clinical evidence of arthritis ('lung-limited' disease or interstitial pneumonia with autoimmune features), clinical RA with lung disease or clinical RA without lung disease. A proportion of individuals with lung-limited RA (interstitial pneumonia with autoimmune features) progress to also develop articular disease and clinical RA. Individuals at risk of developing RA can be targeted with aggressive strategies aimed at smoking cessation and education regarding the potential risks associated with electronic cigarette (e-cigarette) use and other nicotine delivery devices. Individuals with ACPA-positive preclinical RA can be longitudinally followed and screened for lung disease using lung function tests and/or high-resolution chest CT imaging. GERD, gastro-oesophageal reflux disease.

Pulmonary hypertension
A medical condition associated with an elevated pressure (hypertension) in the pulmonary arteries.

Cricoarytenoiditis
Inflammation of the cricoarytenoid joint (a synovial joint located between the arytenoid and cricoid cartilages in the neck), which can occur in rheumatoid arthritis.

Constrictive bronchiolitis
A histopathological term for the bronchiolar (small airway) disorder characterized by fibroproliferative thickening of the bronchiolar walls causing narrowing of the bronchioles.

Follicular bronchiolitis
A bronchiolar disorder associated with bronchiolar narrowing as a result of inflammation and lymphoid hyperplasia of bronchus-associated lymphoid tissue.

Obliterative bronchiolitis
The clinical term used to describe constrictive small-airway bronchiolar diseases that can occur in a variety of clinical contexts, including rheumatoid arthritis; the corresponding histopathological entity to obliterative bronchiolitis is constrictive bronchiolitis.

Pleural effusion
Excessive fluid build-up that happens between visceral and parietal pleura.

Usual interstitial pneumonia
A form of interstitial lung disease associated with a characteristic histopathological pattern on lung biopsy and radiological pattern on chest CT.

Non-specific interstitial pneumonia
A distinct subgroup of interstitial lung disease with characteristic histopathological findings in lung tissue.

Clubbing
A deformity of the fingers and/or toes associated with enlargement of the fingertips and increased curvature of the nails that is associated with a number of lung and other disorders.

As opposed to phenotypes, characterizing subgroups of RA-associated lung disease by endotype would involve the consideration of distinct functional or pathophysiological mechanisms (for example, the relative role of pro-inflammatory cytokines) relevant to that subgroup of RA-related lung disease, which ultimately could have utility in directing therapy or determining prognosis.

Among the different RA-associated lung manifestations, ILD is the most challenging clinically, as it might lead to substantial morbidity and premature mortality[25,33]. ILD in patients with RA often conforms to the usual interstitial pneumonia (UIP) pattern on diagnostic images and tissue samples, unlike other autoimmune diseases such as systemic sclerosis (SSc), in which the most common pattern of ILD is non-specific interstitial pneumonia (NSIP)[24,34,35]. RA-associated NSIP and RA-associated UIP are distinct phenotypes that can be associated with different outcomes; in a retrospective series, the median survival of patients with RA with radiographically defined UIP was worse than for those with NSIP[35,36]. However, to our knowledge, no prospective natural history studies have been performed that compared outcomes in patients with different subgroups of RA-associated ILD.

Screening

Despite the availability of tools to evaluate RA-associated lung disease, such as pulmonary function testing and HRCT, such tests are not routinely performed in patients without symptoms of lung disease. A diagnostic algorithm for the evaluation of RA-associated ILD has been proposed[37], which involves taking a history directed at the detection of shortness of breath on exertion or cough, and a physical examination aimed at detecting pulmonary crackles or clubbing. In the absence of symptoms, such screening would be repeated annually. Although cost-effective and relatively easy to implement, such a screening strategy could lead to an under-recognition of sub-clinical pulmonary disease owing to the limited sensitivity of using symptoms to detect early disease. Arguments have been made against routine pulmonary function testing or HRCT in patients with RA owing to a lack of cost-effectiveness[38]. Another possible screening method is the use of chest radiography at the

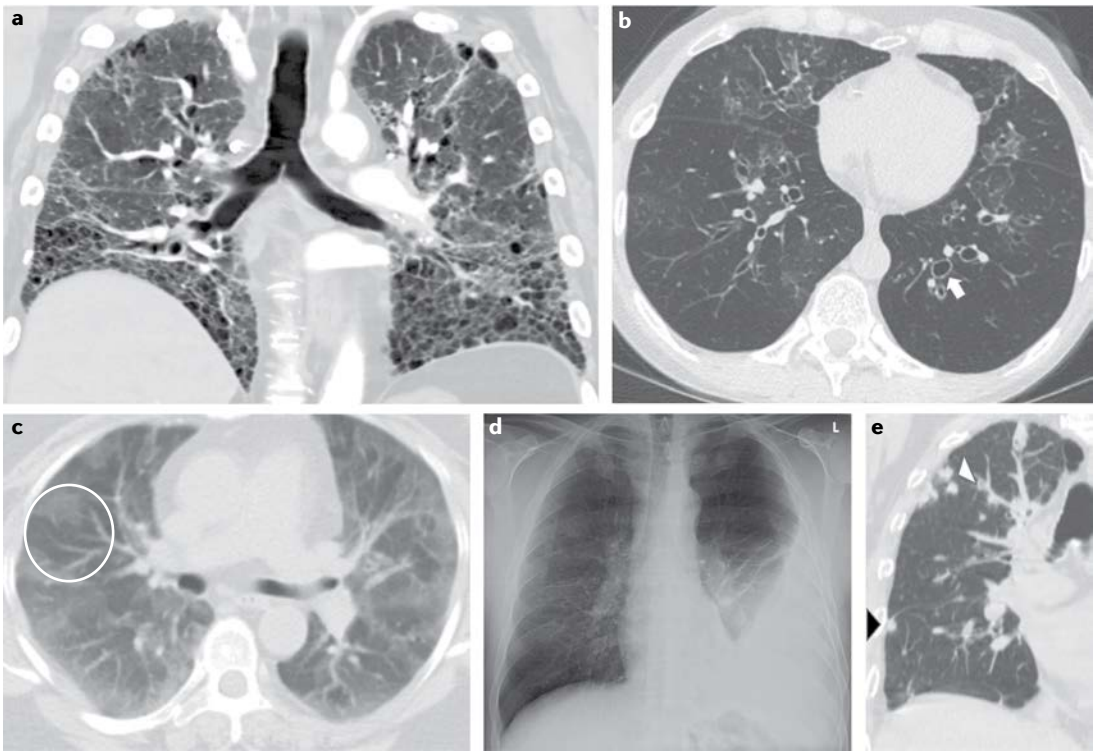

Fig. 2 | **The spectrum of RA-associated lung disease. a** | A CT image of a 54-year-old man with seropositive rheumatoid arthritis (RA) and advanced RA-associated interstitial lung disease (ILD). The representative coronal image shows bilateral, sub-pleural and basilar predominant, reticular linear opacities with prominent associated honeycombing (note how regions of the lower lobe are architecturally deformed and assume a shape reminiscent of a honeycomb). **b** | A representative chest CT image from a 57-year-old woman with long-standing severe seropositive RA and extensive bronchiectasis with bronchial wall dilatation, particularly evident in the lower lobes (arrow points to a particularly prominent and dilated airway). **c** | A representative chest CT image from a 74-year-old woman with seropositive RA and obliterative bronchiolitis. A pattern of varying lung attenuation (so-called mosaic attenuation, consisting of areas of hyper-lucent lung adjacent to more normal-appearing areas of lung attenuation (see highlighted circle)) is present on this expiratory image, consistent with obstructive small airways disease. **d** | A chest radiograph from a 55-year-old man with active RA of recent onset showing a moderate left-sided pleural effusion. **e** | A coronal CT image from a 74-year-old man with seropositive RA showing multiple rheumatoid nodules, most prominently in the upper lobe. The white arrowhead indicates two nodules in the right upper lobe and the black arrowhead indicates a nodule in the right lower lobe.

time of RA diagnosis, which could prompt additional evaluation in appropriate patients[38]. However, chest radiography might also be of limited value in this context owing to its low sensitivity for detecting early interstitial or sub-clinical airway disease. As a substantial proportion of patients with RA are smokers[13], the opportunity to enrol eligible patients in lung cancer screening programmes that include low-dose chest CT should be actively encouraged, as it would also serve the purpose of screening for early or sub-clinical disease in individuals who smoke and have RA.

An alternative screening strategy might include the use of lung disease biomarkers associated with an increased risk of RA-associated ILD or with the development of lung disease. The identification of distinct peripheral blood proteins predictive of the development of specific lung phenotypes in patients with RA would be an important tool for identifying at-risk individuals. Efforts to identify serological biomarkers that reliably predict the presence, severity and risk of progression of RA-ILD have so far not achieved clinical utility. The presence of serum ACPAs or their titre are not useful as markers of risk[39] and, similarly, although the concentrations of the matrix metalloproteinase MMP7, CXC-chemokine ligand 10 (CXCL10) and some heat shock proteins are increased in the serum of patients with RA and ILD (when compared with patients with RA but no ILD), they do not reliably predict the development of ILD or any other lung phenotype[40]. A practical approach to screening for lung disease would be to use HRCT as a screening tool for lung disease in all patients with RA who are current or former smokers and who qualify for lung cancer screening[41], and use annual pulmonary function testing (with or without HRCT) for patients with RA who are current or former smokers, but who do not qualify for lung cancer screening.

Pathogenesis of lung disease in RA

Airway and alveolar epithelial cell injury are probably important early events that precede the development of autoimmunity in the lung (FIG. 3). These cells are the first line of defence to inhaled substances, such as cigarette smoke or irritants, and pathogens, which are potential primary triggers of mucosal injury. Once tissue injury

occurs, normal homeostatic responses promote healing and the restoration of the lung anatomy. However, in individuals with certain genetic backgrounds, with altered mucosal microbial flora or who smoke, persistent antigenic stimulation can lead to failure of tolerance, resulting in an adaptive immune response to self. Although the precise mechanisms involved are unknown, lung dendritic cells (DCs) are probably pivotal in the orchestration of this autoimmune reaction[42]. Understanding how the interactions between environmental factors (such as cigarette smoke), the lung microbiota and host genetics influence tolerance at mucosal surfaces (particularly at the oral and pulmonary mucosae) is necessary for a complete understanding of the pathophysiology of RA-associated lung disease.

Cigarette smoking

Environmental factors, infection, cigarette smoking and host genetics all influence predisposition to articular, as well as pulmonary disease in RA[11]. A central event leading to RA-associated autoimmunity is the breakdown of tolerance to autoantigens, resulting in downstream autoantibody production and T cell activation towards self-peptides. Evidence exists to support a direct link between smoking and seropositive RA[9,13,43–46], suggesting that smoking might be the most important exogenous factor in the development of autoimmunity prior to the onset of RA. For example, smoking a cumulative 41–50 pack-years increases the odds ratio of developing RA to 13.54 (95% CI 2.89–63.38)[13]. Another study showed that patients with RA who smoked >25 pack years were 3.1 times more likely to be positive for rheumatoid factor, and 2.4 times more likely to have joint erosions than patients with RA who did not smoke[12], suggesting that cumulative cigarette smoke exposure might influence the severity of articular manifestations in RA. Cigarette smoking might also be an independent predictor of ILD development in patients with RA[47], although a large 2018 study did not find a statistically significant difference in smoking status between patients with RA and ILD and a matched cohort of patients with RA and no ILD[48]. In addition, cigarette smoking has been associated with the presence of rheumatoid nodules[12], but has not been shown to directly correlate with an increased risk of other pulmonary manifestations.

Cigarette smoking is the primary cause of chronic obstructive pulmonary disease (COPD) and lung cancer in the USA, and also increases the risk of fibrotic ILD in individuals without RA[49]. Therefore, the fact that patients with RA who smoke can develop both airway disease and parenchymal lung disease (as independent lesions or as coexistent lesions in the same patient[50,51]) and also have an increased risk of developing autoimmunity[52] suggests that mechanisms that promote the breakdown of tolerance in the lung and subsequent systemic RA might overlap with those that result in the development of different lung phenotypes. Although COPD and ILD are distinct phenotypes of smoking-induced lung injury, these entities can occur in the same individual and share common pathways of injury that interact with other factors (such as the genetic background of the host) to result in a particular pattern of disease[53]. The extent to which cellular mechanisms, such as cigarette smoking-induced oxidative stress, innate and adaptive immune cell activation and dysregulation, and the influence of epithelial cell turnover and regeneration following injury, might be relevant to the development of particular RA lung phenotypes is unclear; however, these mechanisms are involved in the pathogenesis of analogous patterns or injury in the lungs of individuals without RA who smoke[54,55]. Overall, regardless of whether lung disease is the initial manifestation of RA or RA-associated lung disease is a later extra-articular manifestation, it seems that exposure to cigarette smoke is an important environmental insult that promotes a cascade of events that leads to regional and systemic autoimmunity in individuals with specific genetic backgrounds.

Which cigarette smoke toxin?. Tobacco smoke is a complex mixture of thousands of chemicals. Nicotine, polycyclic aromatic hydrocarbons (PAHs), reactive oxygen species (ROS), heavy metals (such as cadmium) and other compounds found in cigarette smoke can all affect mucosal immunity in complex ways[56]. Among these compounds, PAHs have attracted particular interest. PAHs, including 2,3,7,8-tetrachlorodibenzo-p-dioxin and benzo(a)pyrene (BaP), activate the aryl hydrocarbon receptor (AHR), a ligand-activating transcription factor[57] that is expressed in the synovial tissue of patients with RA[58]. Notably, within the synovial tissue of patients with RA who smoke, AHR activation occurs mainly in a subset of synovial DCs, and in-vitro exposure of DCs with BaP reduced DC activation upon stimulation with polyinosinic:polycytidylic acid and the subsequent production of IL-6[58], suggesting a potential link among cigarette smoke, DCs and inflammation in the RA joint.

The importance of AHR in autoimmune arthritis is illustrated by the fact that AHR-deficient mice are resistant to collagen-induced arthritis (CIA) and have attenuated T helper 17 (T_H17) cell-mediated immune responses[59]. Whether activation of AHR-dependent pathways occurs in immune or stromal cells in the lungs of patients with RA is unknown, but this pathway is a potentially relevant target that might be involved in sustaining persistent T_H17 cell-mediated inflammation in the lung. One study[60] showed that AHR-dependent signalling through downstream arachidonic acid metabolites induces pulmonary fibroblast migration and myofibroblast differentiation, two important events in the establishment of fibrotic lung disease. However, another study showed suppression of the myofibroblast phenotype when orbital fibroblasts were incubated with transforming growth factor-β (TGFβ) in the presence of endogenous AHR ligands[61]. Overall, although direct evidence linking the AHR pathway and RA-associated lung disease is lacking, the indication that cigarette smoke constituents activate the AHR pathway and the potential induction of downstream pro-fibrotic effects and T_H17 cell polarization suggest that this pathway might be involved in the tissue remodelling and fibrosis that occur in the lungs of patients with RA[62,63].

Nicotine is another cigarette smoke constituent that might be important in the induction of RA-associated

Pack-years
Each pack-year is the equivalent of a pack of 20 cigarettes smoked every day for 1 year.

Table 1 | **Phenotypes, risk factors and prevalence of RA-associated lung disease**

Phenotypes	Risk factors or associations	Study population or source type	Prevalence
Parenchymal disease			
ILD (all subtypes)	Male sex, age >50, smoker (>25 pack-years), long disease duration and high anti-CCP antibody and rheumatoid factor titres[19,20,27,47,127]	Patients with RA with or without pulmonary symptoms[151,152]	Radiographic pattern: 19–33%
		Patients with preclinical ILD and asymptomatic patients[153]	Radiographic pattern: 33%
		Patients with RA with or without pulmonary symptoms[152]	• Radiographic pattern: 19–33% • Clinically significant[a]: 14%
		Patients with RA[25]	Clinically significant[b]: 6.8% in women, 9.8% in men
ILD (usual interstitial pneumonia)	Male sex, age >50, smoker (>25 pack-years), long disease duration and high anti-CCP antibody and rheumatoid factor titres[19,20,27,47,127]	Patients with RA-associated ILD with or without pulmonary symptoms[33,114]	Radiographic pattern: 24–29%
		Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Radiographic pattern: 41%
		Patients with RA-associated ILD[38]	Histopathological and radiographic correlation: 89%
		Patients with RA with suspected ILD[24]	Histopathological pattern: 56%
ILD (non-specific interstitial pneumonia)	Male sex, age >50, smoker (>25 pack-years), long disease duration and high anti-CCP antibody and rheumatoid factor titres[19,20,27,47,127]	Patients with RA-associated ILD with or without pulmonary symptoms[33]	Radiographic pattern: 23%
		Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Radiographic pattern: 30%
		Patients with RA-associated ILD[38]	Histopathological and radiographic correlation: 93%
		Patients with RA with suspected ILD[24]	Histopathological pattern: 33%
Bronchiolitis	Male sex, age >50, smoker (>25 pack-years), long disease duration and high anti-CCP antibody and rheumatoid factor titres[19,20,27,47,127]	Patients with RA-associated ILD with or without pulmonary symptoms[155]	Radiographic pattern: 8%
		Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Radiographic pattern: 17%
ILD (organizing pneumonia)	Insufficient data	Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Radiographic pattern: 8%
ILD (lymphoid interstitial pneumonia)	Insufficient data	Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Radiographic pattern: <2%
ILD (diffuse alveolar damage)	Insufficient data	Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Radiographic pattern: <2%
ILD (desquamative interstitial pneumonia)	Male sex, age >50, smoker (>25 pack years), long disease duration and high anti-CCP antibody and rheumatoid factor titres[19,20,27,47,127]	Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Histopathological evaluation: <1%
ILD (combined pulmonary fibrosis and emphysema)	Cigarette smoking[50]	Patients with RA with or without pulmonary symptoms[151]	Radiographic evaluation: 8%
		Patients with RA-associated ILD with or without pulmonary symptoms[51]	Radiographic evaluation: 27%
Rheumatoid nodules	Male sex, smoker, high disease severity and activity, high rheumatoid factor titre and subcutaneous nodules[31]	Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Radiographic evaluation: 49%
		Cohort of patients with RA who underwent CT owing to suspected associated pulmonary disease[31]	Radiographic evaluation: 22%
Caplan's syndrome	High rheumatoid factor titre and exposure to pneumoconiosis[28]	German miners with pneumoconiosis and patients with coal-worker's pneumoconiosis in the USA and Japan[156]	<1% on autopsy

Table 1 (cont.) | **Phenotypes, risk factors and prevalence of RA-associated lung disease**

Phenotypes	Risk factors or associations	Study population or source type	Prevalence
Airway disease			
Cricoarytenoiditis (upper airway)	Female sex[157]	Patients with RA[157–159]	• Indirect laryngoscopy: 32% • Direct fibreoptic laryngoscopy: 75% • Radiographic pattern: 54–72%
Bronchiectasis (dilated lower airways)	Chronic infection[28]	Cohort of patients with RA who underwent CT exam owing to chest symptoms or abnormal CXR[154]	Radiographic pattern: 22%
		Patients with RA evaluated for HRCT features of airway disease in the absence of ILD[26]	Radiographic pattern: 30%
		Patients with RA with combined bronchiectasis and small airways disease[160]	Radiographic pattern: 40%
		Cohort of patients with RA who underwent CT owing to suspicion of associated pulmonary disease[31]	Radiographic pattern: 51%
Constrictive bronchiolitis (small lower airway disease)	Female sex, high rheumatoid factor titre and long disease duration[28]	Patients with RA with or without pulmonary symptoms[155]	By pulmonary function testing or radiographic pattern: 8–30%
Vascular disease			
Pulmonary hypertension	Long disease duration[161]	Patients with RA with PASP >30 mmHg[161]	By echocardiography: 20–26.7%
		Cohort of patients with RA without coexisting cardiopulmonary diseases, PASP >30 mmHg[162]	By echocardiography: 27.5%
		Unselected population of patients with RA, irrespective of cardiopulmonary symptoms, PASP ≥30 mmHg[163]	By echocardiography: 21–31%
Vasculitis	High rheumatoid factor titre[164]	Systematic review of systemic rheumatoid vasculitis[164]	Diffuse alveolar haemorrhage owing to pulmonary capillaritis uncommon
		Cohort of patients with biopsy-proven pulmonary capillaritis with criteria for RA[165]	Diffuse alveolar haemorrhage due to pulmonary capillaritis uncommon
Pleural disease			
Effusion	Male sex, age >35, high disease severity and duration and subcutaneous nodules[30]	Patients with RA with symptomatic pleural effusion[30]	3–5%
Pleurisy	Male sex[166]	Patients with RA[166]	By history and symptoms: 21%
Sequelae of pleurisy (pleural thickening or effusion)	Male sex[166]	Symptomatic pleural effusion in cohort of patients with RA[30]	Radiographic pattern: 24% men, 16% women
Bronchopleural fistula	RA-associated nodules, pneumothorax and eosinophilia[32]	Case report[167]	Infrequent
Pneumothorax	RA-associated nodules and eosinophilia[32]	Case reports[168,169]	Infrequent

CCP, cyclic citrullinated peptide; CXR, chest X-ray; HRCT, high-resolution CT; ILD, interstitial lung disease; PASP, pulmonary artery systolic pressure; RA, rheumatoid arthritis. [a]Defined by symptoms or signs, radiographic changes of ILD on HRCT or CXR, and restrictive lung physiology or abnormal BAL. [b]Defined as being recorded on death certificate as contributor to death process.

autoimmunity. Despite intensive investigation, the role of nicotine in the pathogenesis of both articular RA and RA-associated lung disease remains only partially understood, and the extent to which nicotine contributes to lung disease development in RA remains unknown. Conflicting evidence exists from studies of experimental models of arthritis, in which the treatment of mice or rats with nicotine prior to induction of disease either attenuated arthritis[64] or exacerbated it[65]. The complexity of the effects of nicotine on RA-associated autoimmunity are further illustrated by the results of a study in a rat model of arthritis in which nicotine functioned as both a facilitator and a suppressor of specific aspects of autoimmunity, depending on when the nicotine exposure occurred during the experimental model[66].

Several potential mechanisms exist by which nicotine could promote lung disease in RA. One potential mechanism involves the release of neutrophil extracellular traps (NETs), a process known as NETosis, which is important in the pathogenesis of several autoimmune disorders,

including vasculitis, systemic lupus erythematosus and RA[67]. Nicotine induces NETosis in a dose-dependent manner, which is increased in the presence of ACPAs[65]. Given that NETs display histones that have been citrullinated by peptidylarginine deiminase 4 (PAD4)[68], and that expression of PAD4 is induced by cigarette smoke in mice with CIA[11], it is possible that the airway mucosa is a site where protein citrullination and NETosis co-occur, resulting in the generation of antigenic targets that yield specific ACPAs.

Nicotine can also mediate airway and parenchymal lung injury via damage to the epithelial and endothelial cell barriers, stimulating the production and release of TGFβ, the recruitment of pro-inflammatory cells, the activation of ROS production and the direct promotion of epithelial-to-mesenchymal transition[69–71]. The extent

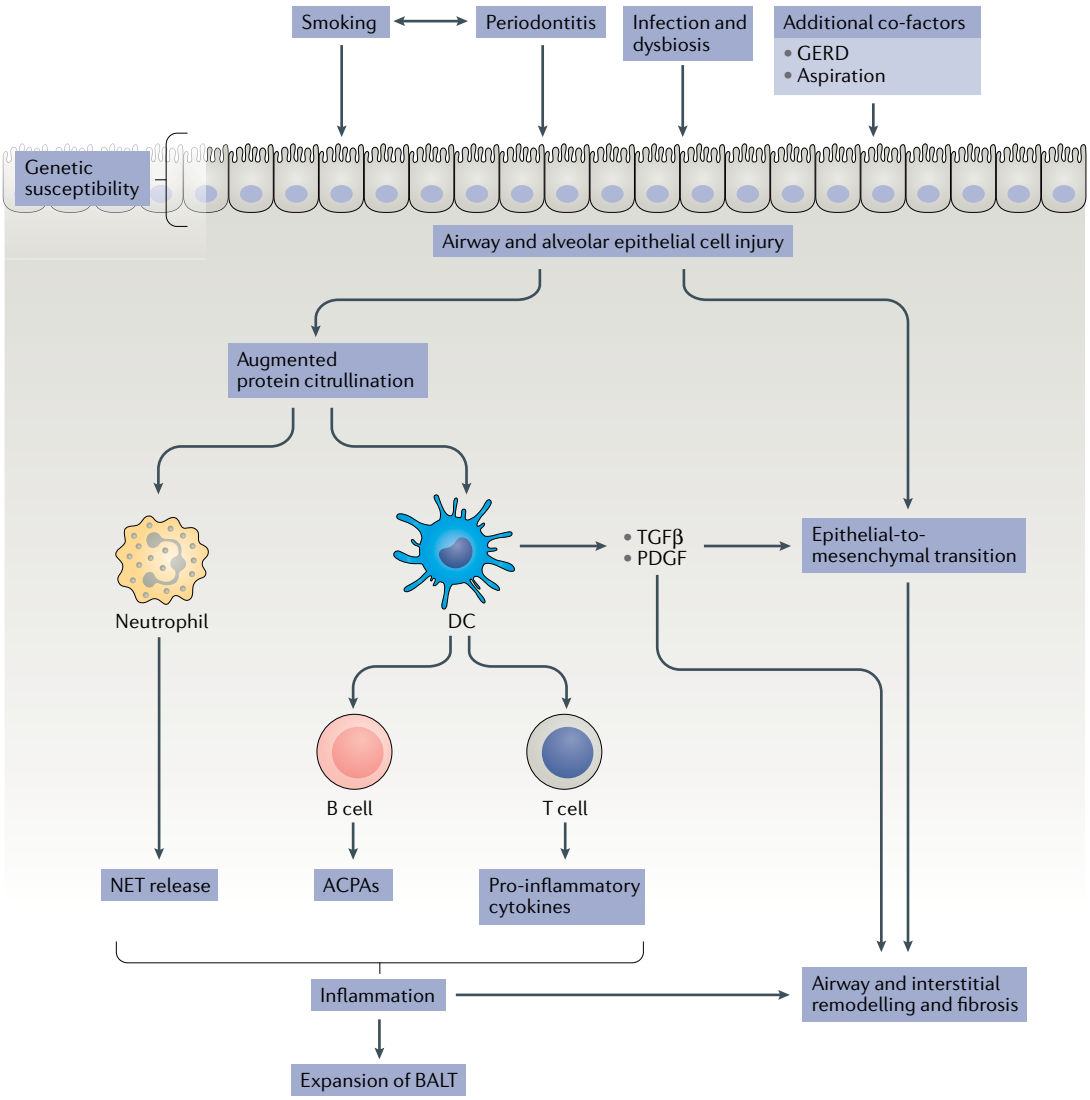

Fig. 3 | **Proposed mechanism of RA-associated lung disease development.** The first step in the induction of rheumatoid arthritis (RA)-associated lung disease involves the occurrence of airway and/or alveolar epithelial cell injury in individuals with predisposing genetic backgrounds, particularly in the context of tobacco use and exposure to environmental factors that cause oxidative stress or infection. Aspiration of saliva with *Porphyromonas gingivalis* (or regurgitation of gastric contents in individuals with gastro-oesophageal reflux disease (GERD)) might also be involved in airway mucosal injury. Persistent or repetitive injury to the airway mucosa or distal lung structures activates the innate immune system leading to an inflammatory response. Increased protein citrullination occurs owing to augmented peptidylarginine deiminase activity. In some individuals, particularly those with certain genetic backgrounds, immune tolerance fails and an autoimmune response is triggered, resulting in the generation of RA-associated anti-citrullinated protein antibodies (ACPAs). The ensuing inflammation results in the development of inducible bronchus-associated lymphoid tissue (iBALT), particularly near airways, perivascular spaces and interstitial regions. This autoimmune response is initially localized to the lungs, but upon further injury it can be amplified and subsequently develop into a systemic autoimmune process. Activation of local immune cells such as macrophages and dendritic cells (DCs), as well as repetitive epithelial cell injury, results in the release of transforming growth factor-β (TGFβ) and other cytokines, such as platelet-derived growth factor (PDGF), that promote epithelial-to-mesenchymal transition and airway and distal lung remodelling and fibrosis. Immune cells in the lung further amplify the autoimmune response, fibrosis and tissue remodelling. NET, neutrophil extracellular trap.

to which these nicotine-mediated effects occur in the lungs of patients with RA who smoke or use nicotine (either systemically via patches or inhaled via electronic cigarettes) is unknown and it is possible that cigarette smoke constituents other than nicotine are primarily responsible for the induction of RA-associated lung disease. However, given the widespread use of nicotine, and particularly regarding the increased use of electronic cigarettes, further investigation into the specific effects of nicotine on the development of RA-associated lung disease is clearly warranted.

Cigarette smoke and DCs. The pathogenesis of RA is widely believed to involve a dynamic relationship between T cell-mediated adaptive immune response and 'upstream' events related to the activation of innate immune cells. The regulation of T cell activation in the lung is reliant on DCs, important mediators of adaptive immune responses and immune tolerance, functions that depend on a variety of factors, including the expression of stimulatory and inhibitory ligands and chemokine receptors, the presence of soluble mediators and other factors[72]. Given that a clear relationship exists between cigarette smoking and RA (and potentially the development of RA-associated lung disease), and considering the primacy of mucosal DCs in regulating adaptive immune responses, it is crucial to examine the effects of cigarette smoking on lung DCs in the context of RA.

Cigarette smoking affects DC function in a multitude of ways that might be relevant to the development of autoimmunity in the lung[73–76]. To start with, when compared with non-smokers, individuals who smoke have increased numbers of DCs in both the airways and interstitial lung compartments[75]. A study in mice showed that cigarette smoke inhalation delays the development of tolerance to exogenous antigens[77], and another study showed that cigarette smoke suppresses tolerogenic responses by plasmacytoid DCs[78]. Cigarette smoke also induces the production of a variety of epithelial cell-derived cytokines that could influence local DC responses in the lung. Some of these cytokines, such as TGFβ[79], can suppress DC maturation and promote tolerance (although in the presence of IL-6 and/or IL-23, TGFβ can also promote T_H17 cell polarization), whereas other cytokines, such as granulocyte–macrophage colony-stimulating factor[80] and thymic stromal lymphopoetin[81], can enhance and skew DC maturation, thereby favouring inflammation and autoimmunity in the appropriate contexts.

Important insights into the potential role of lung DCs in the development of RA-associated lung disease were provided by a 2017 study[82] in SKG mice, which develop arthritis and diffuse lung inflammation after a single injection of the carbohydrate zymosan-A. The authors identified a new type of lung DC (characterized as $CD11b^{+}Gr\text{-}1^{dim}$) with tolerogenic properties in the inflamed lungs of SKG mice[82]. Adoptive transfer of these tolerogenic DCs suppressed lung inflammation in zymosan-A-treated SKG mice[82], implicating a direct role for DCs in suppressing lung inflammation in this model. Another important observation that suggests a central role for lung DCs in the generation of RA-related autoimmunity, as well as RA-associated lung disease, comes from a study that showed the presence of lymphoid follicles in a predominantly peribronchiolar distribution in lung tissue from patients with RA[83]. These tertiary lymphoid follicles are known as inducible bronchus-associated lymphoid tissue (iBALT), and are induced in the lung in response to chronic antigen exposure[84]. A study that evaluated immune cell infiltrates in iBALT from patients with RA-associated ILD and patients with idiopathic ILD reported prominent T cell and B cell infiltrates in tissues from both patient groups: however, follicular DCs were prominent only in tissue from patients with RA-associated NSIP or UIP[83]. Follicular DCs are particularly relevant in this context in the iBALT structures of RA-ILD owing to their role in high-affinity antibody production and in the development of B cell memory, two important biological processes in RA-related autoimmunity[85]. Whether cigarette smoking directly affects follicular DC function is not well established. However, the increases in the number of myofibroblasts and in collagen production that occur in close proximity to iBALT in RA-ILD[83] suggest a potential role for iBALT in the induction of myofibroblasts, as well as in RA-related autoimmunity.

Gene–environment associations

The effects of smoking on the pathogenesis of RA differ depending on the genetic background of an individual, particularly their HLA genes (TABLE 2). HLA-DRB1*04:01 was the first HLA molecule to be associated with a predisposition to RA[86]. Similar sequences located at positions 70 and 74 of the third hypervariable region of the HLA-DRB1 molecule — the so-called shared epitope — promote the presentation of certain RA-associated antigens, such as citrullinated vimentin[14]. An increased risk of RA as a result of smoking mainly occurs in individuals who are positive for the shared epitope[87]. In addition, the effect of the shared epitope on the risk of RA is proportional to the number of copies present; when two copies of the shared epitope are present, the relative risk of RA in individuals who smoke (compared with individuals who do not smoke and do not have any shared epitope genes) increases by 15.7 times[88].

Different *HLA-DRB* alleles have different effects on the risk of RA; individuals who are positive for *HLA-DRB1*0401* and smoke have the highest rate of rheumatoid factor positivity, estimated at 3.7 times that of a non-smoker who is *HLA-DRB1*0401*-negative[89], whereas having *HLA-DRB1*01:01*, *HLA-DRB1*01:02* or *HLA-DRB1*10:01* alleles increases the risk of ACPA positivity[90]. Notably, in studies conducted in predominantly white populations, neither smoking nor shared epitope genes (nor a combination of these factors) influenced the risk of developing seronegative RA, implying that smoking and shared epitope genes are relevant only to the induction of seropositive disease, thereby affirming the importance of this gene–environment association in the induction of RA-associated autoimmunity[88].

The effect of smoking on lung inflammation in mice with CIA was also highly dependent on genetic background[11]. The interaction between host genes and smoking was explored using HLA transgenic mice, in

Table 2 | **Genetic variants associated with RA-associated lung disease**

Gene	Genetic variant	Association	Refs
HLA genes	*HLA-DRB1* shared epitope	Associated with a reduced risk of ILD	96
	*HLA-DQB1*03:01*	Associated with a predisposition to bronchiectatic airway disease or emphysema	97
	*HLA-DBQ1*03:02*	Associated with reduced likelihood of developing bronchiectatic airway disease or emphysema	97
	*HLA-DRB1*15* and *HLA-DRB1*16*	Associated with an increased risk of ILD	96
MUC5B	rs35705950 G>T	• Gain-of-function promoter variant • Genetic risk factor for IPF • Associated with RA-ILD compared with controls (patients with RA but no ILD as well as healthy individuals)	48,130
SFTPC	• c.180 G>A, p.Met60Ile • c.218 T>C, p.Ile73Thr	Associated with diffuse parenchymal lung disease and alveolar type 2 cell dysfunction	136,137
RTEL1	• c.900 C>G, p.Ser300Arg • c.2695 T>C, p.Phe899Leu • c.2824 G>A, p.Asp942Asn • c.2875 C>T, p.His959Tyr • c.2890 T>C, p.Phe964Leu	Coding region mutation leading to telomere shortening and onset of RA-associated ILD at a younger age	136
TERT	• c.1234 C>T, p.His412Tyr • c.2383-2 A>G • c.3323 C>T, p.Pro1108Leu	Coding region mutation leading to telomere shortening and onset of RA-associated ILD at a younger age	136

ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; RA, rheumatoid arthritis.

which endogenous murine MHC class II molecules were replaced by human HLA-DR and HLA-DQ molecules. When exposed to cigarette smoke and subsequently challenged with collagen and adjuvant systemically, the mice developed lung inflammation, systemic arthritis and systemic ACPAs analogous to human RA[11]. In this model, the effect of smoking on inflammatory cytokine genes in the lungs was dependent on the genetic background of the mice, with statistically significant induction in cytokine gene expression observed in HLA-DQ8-positive but not HLA-DR4-positive mice[11]. Furthermore, exacerbation of arthritis by cigarette smoke was only evident in HLA-DQ8-positive mice and correlated with increased expression of *PAD2* and *PAD4* in the lungs of mice exposed to cigarette smoke[11]. The results of this study suggest that dysregulation of PAD enzymes in the lung might be an important molecular checkpoint that links environmental stressors (such as cigarette smoking and infection), host genes and the development of autoimmunity.

Consistent with evidence from mouse models of arthritis[11,14], a study performed on otherwise healthy individuals who smoke reported increased expression of PAD2 in both proximal and distal airways, as well as increased concentrations of citrullinated peptides in BAL cells, relative to a matched cohort of healthy non-smokers[91]. PADs might be particularly relevant to the pathogenesis of RA in individuals with polymorphisms in *PTPN22*, which encodes a haematopoietic-specific protein tyrosine phosphatase[92,93]. Normally, PTPN22 inhibits PAD4 and downstream protein citrullination; however, because the PTPN22 (R620W) variant is deficient in its inhibitory function, citrullination of proteins is enhanced in individuals carrying this variant[94]. Whether this polymorphism is associated with lung disease in RA is not known, but a 2018 study showed that the same *PTPN22* polymorphism (rs2476601) is an independent risk factor for obliterative bronchiolitis (a constrictive small airway disease that also occurs in RA) in lung transplant recipients[95].

It is important to mention a number of other genetic associations in the context of specific phenotypes of RA-associated lung disease that are not necessarily linked with smoking or other environmental exposures. The role of HLA genes in RA-associated lung disease has been investigated in a cohort of Japanese patients with RA[96,97]. In this population, the *HLA-DRB1* shared epitope was associated with a reduced risk of ILD, whereas the *HLA-DR2* alleles (including *HLA-DRB1*15* and *HLA-DRB1*16*) were associated with an increased risk of ILD[96]. Predisposition to bronchiectasis or emphysema in RA was associated with *HLA-DQB1*03:01*, and *HLA-DQB1*03:02* was associated with a low incidence of these lung manifestations[97]. The difference in genetic predisposition to ILD and airway disease in patients with RA was also investigated in a cohort of Japanese patients[47]. In this study, the *HLA-DRB1*15:01* allele was positively associated with ILD, but negatively associated with airway disease, supporting the idea that different genetic predispositions exist for specific phenotypes of RA-associated lung disease.

Mutations in *COPA* that cause autoimmune-mediated ILD and arthritis also provide a compelling insight into potential mechanisms of disease that could translate to RA-associated lung disease[98,99]. Unique variants of *COPA* cause aberrant intracellular transport resulting in endoplasmic reticulum (ER) stress that leads to T_H17-mediated autoimmune responses[99]. This mechanism is of interest because cigarette smoke is a potent inducer of ER stress[100], hinting that cigarette smoke-induced ER stress in the lung might also be of relevance to the induction of autoimmunity, T_H17 cell polarization and diffuse lung disease in RA.

Emphysema
A lung disorder associated with the destruction of alveolar units that clinically results in shortness of breath and exercise limitation.

Dysbiosis
An altered microbiome composition linked with the transition from healthy mucosal tissue to a state of dysfunction.

Oral and airway dysbiosis

The tracheobronchial tree and lung parenchyma are not sterile sites, but rather harbour a complex and diverse microbiome, the composition of which is modifiable by disease, as well as by smoking, antibiotic therapy and many other exogenous and endogenous factors. Perturbations in this microbiota promote a state of dysbiosis, which can occur in the oral cavity and lungs, as well as in the gut, of patients with RA[101–104]. Such alterations in the microbial ecology at multiple mucosal surfaces might be crucial for the initiation and subsequent progression of autoimmunity in predisposed hosts. The generation of mucosal IgA in the lung is substantially influenced by microbiota, and germ-free mice generate reduced amounts of protective IgA[105], suggesting an important role for the microbiome not only in the development of disease but also in the generation of robust protective immunity at mucosal surfaces. However, whether the lung microbiota is influenced by RA-associated genetic factors, as occurs in the gut[106], is unknown. Studies in mice highlight the importance of crosstalk between the gut and lung mucosal microbial communities and demonstrate the important interactions that take place between the lung and gut microbiomes[107]. Although these interactions might provide some insight into the generation of a preclinical autoimmune response in the lungs, as well as the occurrence of lung involvement in a subset of seropositive patients, the mechanism by which lung microbiota can promote or modulate systemic and articular inflammation remains poorly understood.

Most studies on the involvement of endogenous microorganisms in RA have focused on the gut–joint axis, and relatively limited information exists on the role of lung microbiota in the initiation or progression of arthritis. A study in which microbial diversity was compared in BAL fluid from patients with untreated RA and healthy individuals showed a reduction in microbial diversity in patients with RA[103]. The dysbiosis observed in distal airways included a reduced abundance of *Actinomyces* and *Burkholderia* species and an increase in operational taxonomic units belonging to the genus *Pseudonocardia*. Interestingly, the lung microbiomes from individuals with RA and individuals with sarcoidosis (a granulomatous disease not associated with classic autoimmunity or ACPA generation) were similar except for the presence of *Pseudonocardia*, which was associated with increased disease activity in RA only. In this study[103], *Porphyromonas gingivalis*, which has been associated with periodontitis in individuals who smoke and in patients with RA[108], occurred at a lower abundance in BAL fluid from patients with RA than in BAL fluid from healthy individuals. This result is in contrast with those from a mouse model of arthritis in which *P. gingivalis* given orally enhanced disease severity via modulation of the intestinal microbial profile and immune system[109], and intraperitoneal infection caused an increase in citrullinated proteins[110] (*P. gingivalis* has PAD enzymes that can citrullinate human proteins in vivo and in vitro[91]) implying a potential intrinsic mechanism by which this pathogen can augment citrullination at mucosal sites.

The augmented propensity to autoimmunity associated with the presentation of citrullinated proteins by RA-associated HLA molecules suggests a mechanism that links host genetics and specific pathogens[14,106,109]. The association between smoking and *P. gingivalis* suggests another link by which smoking and host factors can promote seropositive RA, linking the oral mucosal microbiome with the development of autoimmunity. Another factor that might contribute to lung dysbiosis in RA is the occurrence of gastro-oesophageal reflux disease (GERD) and the regurgitation of gastric contents into the airways. GERD is increasingly believed to have an important role in the development and progression of fibrotic ILD[111,112]. GERD might potentially provide another mechanism by which previously swallowed saliva containing *P. gingivalis* could be regurgitated and aspirated into the lungs, thereby causing airway mucosal injury and introducing the pathogen into the lower airway. In turn, these changes might promote an increase in protein citrullination and ACPA formation, as found in BAL fluid from patients with RA[91].

ACPAs

Although ACPAs are useful clinically as biomarkers for seropositive RA, their direct role in mediating pathogenesis, particularly at extra-articular sites, is only partially understood. Elucidating the specific role of ACPAs in mediating articular or extra-articular disease is important as it could provide the rationale for antibody depletion (or neutralization) as a direct therapeutic approach for the treatment of specific RA manifestations. Notably, airway and interstitial lung involvement are not restricted to ACPA-positive individuals but also occur in seronegative patients with RA[113,114]. Up to 80% of patients with RA have autoantibodies against cyclic citrullinated peptides that are detectable by commercially available autoantibody assays[115]. However, it is possible that an inability to detect autoantibodies against citrullinated peptides in some patients with seronegative RA is the result of the relatively limited specificities of autoantibodies measured in such assays. A further assumption is that ACPA activity in the lung is reflected by serological status, which is not certain; lung tissue samples are rarely studied and, in those that have been evaluated, the lung and serum are not always concordant with regard to ACPA status[116].

A potential role for ACPAs as mediators of lung disease has been suggested by the results of studies that found a correlation between increased serum ACPA concentrations and RA-associated airway disease[47]. In addition, signs of immune cell accumulation and activation have been found in bronchial tissue and BAL fluid from patients with ACPA-positive RA who do not have clinically evident lung disease[5]. These observations support the idea that the lung is a primary site of ACPA generation, but could also support the alternative perspective that the lung itself is an early target of ACPA-induced injury. Likewise, the presence of chronic airway disease and dysbiosis could either be a primary inducer of ACPA generation and inflammation, or a manifestation of ACPA-mediated lung injury.

Diffusing capacity for carbon monoxide measurement
A physiological parameter of gas transfer efficiency in the lungs.

Other studies have reported a correlation between ACPA concentrations and a reduction in physiological markers of interstitial lung abnormality such as the diffusing capacity for carbon monoxide measurement, implying that patients with RA who have high concentrations of ACPAs are more likely to develop ILD[18]. ACPA titres are substantially higher in individuals with RA-associated ILD than in patients with RA and no ILD[117,118] and an expanded ACPA repertoire (defined as the detection of ≥7 ACPAs at high concentrations) correlates with imaging features consistent with fibrotic ILD[118]. The number of detected ACPAs also correlated more strongly with UIP than with NSIP[118], implying a potential pathogenic role for ACPAs in RA-associated UIP.

Although high ACPA concentrations correlate with more severe articular disease and joint destruction[119], as well as with airway disease and ILD[47,117,118], it is still not clear whether these high concentrations of ACPAs are an epiphenomenon reflecting increased mucosal inflammation and autoantibody generation in individuals with lung disease and RA, or a reflection of how important B cell-mediated injury is in the development of RA-associated lung disease. ACPAs can cause injury to the lung mucosa, airways and interstitium through several potential mechanisms. ACPAs can form immune complexes and activate cells by binding to Fc receptors, resulting in the release of pro-inflammatory cytokines such as IL-6, IL-8 and TNF[120]. In vitro studies have revealed how ACPAs can mediate neutrophil cell death and NETosis, which can further promote inflammation and autoimmunity by releasing citrullinated autoantigens[121]. However, to what extent these mechanisms are relevant in the induction of lung disease in RA remains unclear at this time. B cell-depleting approaches to the treatment of RA-associated ILD have not shown definitive benefits[122–124], although patient numbers in these studies were small, and the studies were not prospective or randomized. In addition, B cell-depleting strategies used in the management of autoimmunity rely on therapeutic schedules that result in peripheral B cell depletion, which might not necessarily correlate with the depletion of autoantibody-producing B cells in the lung.

Similarities to IPF

RA affects the lung in various ways, resulting in patterns of disease analogous to airway and interstitial disorders that occur in the absence of RA. For example, in the most common type of RA-associated ILD, lung tissue usually has a UIP pattern[24,38], which can be indistinguishable by light microscopy or chest HRCT from the same pattern in lung tissue from patients with idiopathic pulmonary fibrosis (IPF). The striking similarity between these autoimmune and idiopathic forms of UIP suggests possible shared pathways of pathogenesis and mechanisms of fibrosis.

IPF and RA-associated ILD share several demographic and clinical features. Cigarette smoking is a recognized predisposing factor for both diseases[125,126], and both disorders occur in older adults, with a mean age of presentation of >55 years of age[127,128]. HRCT image analysis of the lungs of current or former smokers with either IPF or RA-associated ILD revealed the coexistence of radiographically evident emphysema in ~50% of individuals with RA-associated ILD and 35% of those with IPF[50]. These results are important, as they highlight the frequent coexistence of emphysema and fibrotic lung injury in RA-associated ILD and in IPF, again not only suggesting common factors involved in disease predisposition (including cigarette smoking and other inhalational injuries), but also common mechanisms of disease development.

Shared genetic risk factors

The mucoprotein MUC5B is secreted by submucosal mucinous gland cells and supports mucosal ciliary function, regulates local immune responses and influences alveolar regeneration following injury[129]. A specific variant of the *MUC5B* promoter (rs35705950) is the strongest genetic risk factor for IPF[130], and has also been described as a strong risk factor for RA-associated ILD, especially in patients with a UIP pattern of disease[48]. Interestingly, the same *MUC5B* polymorphism that predisposes individuals to the development of IPF is also associated with substantially improved survival[131] and a slower decline in lung function over time in patients with IPF[132]. Critical mechanistic insight into the role of MUC5B in fibrotic lung disease comes from studies using transgenic mice that overexpress MUC5B in either conducting airways or distal lung structures[129]. Mice that overexpress MUC5B distally in the lung develop worse lung fibrosis when challenged with bleomycin (one of the most extensively used experimental models of pulmonary fibrosis)[129]. Although polymorphisms in *MUC5B* are associated with an increased risk of RA-associated ILD, no association exists with systemic RA, SSc-associated pulmonary fibrosis or myositis-associated ILD[133–135]. The relevance of the same *MUC5B* polymorphism, a strong risk factor for both RA-ILD and IPF but not for other forms of autoimmunity related lung fibrosis, further supports the proposition that RA-ILD is more closely related to IPF with respect to pathogenesis rather than other forms of pulmonary fibrosis associated with SSc or other connective tissue diseases

Mutations in surfactant-related proteins, particularly surfactant protein C, have also been linked to both IPF and RA-associated ILD[136]. Surfactant protein C is produced by type 2 alveolar epithelial cells, and the association of *SFTPC* mutations with both RA-associated ILD and IPF suggests that focal alveolar injury might occur in these patients as a result of endogenous type 2 alveolar cell dysfunction caused by abnormal surfactant protein processing[137]. Mechanistically, aberrant surfactant protein C processing results in macro-autophagy and secretion of pro-inflammatory and pro-fibrotic mediators by type 2 alveolar epithelial cells, resulting in inflammation, parenchymal injury and fibrosis[137].

Shared immune mechanisms

Although there are many similarities at the macro level between RA-associated ILD and IPF, several important differences also exist at the cellular and molecular levels. Lung tissue from individuals with RA-associated ILD

has substantially greater numbers of B cells and $CD4^+$ T cells than lung tissue from individuals with idiopathic UIP, implying that immune dysregulation might be more prevalent in RA-associated ILD than in idiopathic UIP[138]. Evidence exists for both T_H1 cell-mediated and T_H17 cell-mediated immune responses in RA, and a 2019 study has provided evidence for a role for T_H17 cell-mediated immunity in the pathogenesis of murine pulmonary fibrosis, as well as in RA-associated ILD and IPF[139]. Mechanisms by which T_H17 cytokines such as IL-17A and TGFβ1 cause fibrosis involve direct effects on fibroblasts, leading to their proliferation and extracellular matrix generation[139,140]. In addition to mediating direct pro-fibrotic effects, TGFβ1 is an important cofactor in the generation of T_H17 cells[141], which are increasingly recognized as mediators of both articular and extra-articular aspects of RA pathogenesis[139,142]. The prototypic T_H17 cell cytokine, IL-17A, also has an important role in mouse models of pulmonary fibrosis[143], and its primary receptor IL-17RA is upregulated in both RA-associated ILD and IPF, although the magnitude of expression of IL-17RA is substantially higher in RA-associated ILD than in IPF[139]. Characterization of the roles of T_H17-associated and other specific cytokines in the pathogenesis of RA-associated ILD might provide the insight necessary for the design of future clinical trials using targeted anti-cytokine therapy.

Cell senescence

The fibroblast is central to the development of RA-associated ILD, RA-associated airway fibrosis and IPF. When grown in culture, fibroblasts have a finite replicative potential as a result of the ever decreasing amount and length of telomeric DNA with serial passage[144]. Telomeres comprise repetitive DNA sequences located at the terminal regions of chromosomes and perform the essential function of ensuring chromosomal and genome integrity[145]. Telomeres shorten following repeated rounds of DNA replication, to the point when telomere length reaches a critical threshold that is associated with activation of the DNA damage response, leading to either cell death or cell senescence[145]. In individuals with IPF, cell senescence markers are detectable in epithelial cells and fibroblasts, indicating the activation of senescence pathways in this disease[146]. Importantly, depletion of senescent cells in a murine model of pulmonary fibrosis improved lung function, implying an active role of senescent cells in pulmonary fibrosis[146]. Senescent fibroblasts also secrete a variety of cytokines, chemokines, matrix remodelling proteases and growth factors, all of which have the potential to signal in a paracrine fashion and promote tissue remodelling and fibrosis[146]. Whether similar senescence pathways are active in the lungs of patients with RA-associated ILD has not been directly demonstrated, but a 2017 study showed a higher than expected mutation frequency for telomere maintenance genes including *TERT*, *PARN* and *RTEL1* in patients with RA-associated ILD[136]. These results[136] suggest that in a proportion of patients with RA, premature senescence might have a direct role in the development of lung remodelling and fibrosis. Defective telomerase activity in circulating $CD4^+$ T cells (caused by abnormalities in *TERT*) might also have a direct role in the development of RA-associated lung fibrosis or emphysema[147]. The extent to which interactions between senescent immune and stromal cells promote lung remodelling in RA is not known, but promises to be an area of fruitful investigation. The observation that RA-associated ILD most commonly occurs in individuals >55 years old (as observed with IPF)[148] further supports the contention that senescence pathways might mediate lung disease in patients with RA.

Therapy and prevention

The importance of understanding the pathogenesis of lung disease, as well as acknowledgement of the heterogeneity of RA-associated lung disease, cannot be overstated. Both parenchymal and airway disease in patients with RA present substantial challenges, as available therapeutics do not have a clearly beneficial effect on the prevention, or disease course, of either phenotype. Careful characterization of the relevant phenotypes and endotypes of RA-associated lung disease provides an opportunity for primary prevention of lung disease in individuals at risk, especially individuals with early RA who smoke, or those with a family history of RA who smoke. In addition to encouraging individuals at risk of RA to stop smoking (and to limit exposure to second-hand smoke), all individuals at risk of RA should also be encouraged to avoid using electronic cigarettes (FIG. 1). Although electronic cigarettes are theoretically safer than cigarettes, they are highly effective nicotine delivery devices that could potentially promote RA and/or RA-associated ILD in at-risk individuals. Whether steps to modify the lung, oral or gut microbiome will be fruitful in mitigating RA-associated lung disease development and/or progression remains uncertain, but is another promising area of investigation. Similarly, the effects of acid-suppressing medication or GERD management via medical or surgical approaches on the development of RA-associated lung disease are unknown.

Current treatment strategies for RA-associated ILD focus on supportive measures, including age-appropriate vaccination and smoking cessation, as well as empiric immunosuppressive therapies. Clinical trials designed to determine the effect of the anti-fibrotic agents pirfenidone and nintedanib on lung function decline in patients with RA-associated ILD are ongoing[149,150]. Whether the results are positive or negative, findings from these trials will be important in paving the way for future therapeutic approaches. Novel biomarkers for RA-associated lung manifestations are clearly needed, whether they be blood or serum markers, genetic tests, novel imaging techniques or high sensitivity approaches to detect individuals with early stages of disease, even when lung function is preserved. A better understanding of the biology and natural history of lung complications in RA will guide future efforts to use immunosuppressive and other therapies to treat RA-associated lung disease, perhaps including a multimodal therapy combining anti-fibrotic agents and immunomodulatory agents that target specific pathways relevant to the development of RA-associated lung disease.

Conclusions

RA can affect virtually every lung compartment, causing substantial morbidity and mortality owing to shortness of breath and coughing and, ultimately, respiratory failure and premature death. Lung disease can also predate the onset of articular manifestations of RA by many years. Identifying individuals with RA at risk of developing lung complications is now feasible and should be a goal of rheumatology and pulmonary practices. Screening programmes should particularly target individuals with a family history of RA and patients with early RA who smoke or use electronic cigarettes. Investigations into the specific mechanisms by which lung disease develops in RA are still needed to improve our understanding of RA-associated lung disease, as well as the role of the lung in the initiation and subsequent propagation of systemic autoimmunity. Understanding specific mechanisms that cause airway and distal lung remodelling in RA will be crucial for the development of new pharmacological strategies for treating these extra-articular manifestations of RA.

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Author contributions

D.W., J.Z., J.L. and R.V. researched data for this article. All authors provided substantial contributions to discussions of content and wrote this article. D.W., J.Z., J.L., E.L.M. and R.V. reviewed and/or edited the manuscript before submission.

Competing interests

V.T. declares that she has received research funding from Evelo Biosciences and Jansen Biotech for research unrelated to this Review. E.L.M. declares that he has served as an adviser to Boeringher-Ingelheim (<$10,000). R.V. declares that he has received grant funding from Bristol-Myers-Squibb, Pfizer and Sun Pharmaceuticals. R.V. is also an investigator on a multicentre clinical trial funded by Genentech into rheumatoid arthritis-associated interstitial lung disease (TRAIL-1). The other authors declare no competing interests.

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Publisher's note

Rheumatic manifestations of chikungunya: emerging concepts and interventions

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Abstract | The largest epidemic ever recorded for chikungunya, a disease caused by infection with the chikungunya virus (CHIKV), began in Africa in 2004 and spread to >100 countries on four continents. The epidemic caused >10 million cases of often debilitating rheumatic disease, classically involving rapid onset of fever and polyarthralgia, often with polyarthritis. The clinical diagnosis of chikungunya is often complicated by infections with dengue or Zika virus. For many individuals with chikungunya, the disease is benign and self-limiting; however, some patients have a complex spectrum of atypical and severe manifestations. Many patients also experience a chronic phase of the disease, primarily involving arthralgia (which can be protracted (>1 year)), and a number of sequelae are also recognized. CHIKV-induced arthropathy arises from infection of multiple cell types in the joint and the infiltration of mainly mononuclear cells. Innate responses (primarily involving type I interferon responses and natural killer cells) and cognate responses (primarily involving CD4 T helper 1 cells), alongside activation of macrophages and monocytes, mediate CHIKV-induced arthritic immunopathology. Ideally, improved anti-inflammatory treatments should not compromise antiviral immunity. New concepts in mosquito control are being field tested and a number of CHIKV vaccines are being developed.

Pandemic
An epidemic of disease that has spread across a large region; for instance, multiple continents, or even worldwide.

Attack rate
The total number of new cases of a disease divided by the total population (that is, the percentage of a defined population that is affected by a disease).

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Chikungunya virus (CHIKV) is a member of a group of globally distributed, mosquito-transmitted arthritogenic alphaviruses that cause sporadic outbreaks of primarily rheumatic disease every 2–50 years[1–4]. The largest epidemic of CHIKV disease (hereafter simply referred to as chikungunya) ever recorded began on Lamu Island, Kenya, in 2004 (FIG. 1). The epidemic expanded across four continents, with cases still being reported in 2019 (FIG. 1; Supplementary Table 1). Three major genotypes of CHIKV are now recognized — the Asian, the West African and the Asian and East–Central South African (ECSA) genotypes[5] — but a new lineage, the Indian Ocean Lineage (IOL), also emerged from the ECSA genotype during the 2004–2019 epidemic[6]. The epidemic reached >100 countries (Supplementary Table 1), caused >10 million cases (Supplementary Table 2), and might arguably be called a pandemic. An estimated 1.3 billion people are at risk of chikungunya[7]. Climate change modelling suggests that many more areas of the world (including parts of China, sub-Saharan Africa, South America and the United States) might become able to accommodate transmission of CHIKV in the future[8,9].

Chikungunya was previously often viewed as (and for many patients remains) a relatively benign and self-limiting rheumatic disease. However, a considerably more complex spectrum of less common atypical and severe manifestations is now recognized in subgroups of patients, with chikungunya often complicated by comorbidities and co-infections. Hospitalization rates for chikungunya range from 0.6% to 13%[10–14] and estimates of chikungunya-related mortality range from 0.024% to 0.7%[10,12,15–17]. In addition, many patients develop protracted rheumatic disease lasting many months, occasionally years, with a number of sequelae now also recognized[18–22]. Estimates for the total economic costs (direct and indirect) of chikungunya have ranged from a median of US$67 for adults and $258 for children in Columbia[23], to a mean of $150 per outpatient and $3,300 per inpatient in 2006 in Réunion Island[11]. In a large study of a chikungunya outbreak in Bangladesh in 2017, >10 days of productivity were lost in ~30% of patients with chikungunya because of severe arthropathy[24]. Such costs might be viewed as relatively modest by Western standards; however, the occasionally high attack rate of chikungunya, with up to 30–75% of a given population affected by chikungunya disease at any one time[1,25], can result in a substantial economic burden, especially in resource-poor communities that are often affected by the disease[26].

Considerable research in patients and animal models has now provided extensive insights into the complex

Key points

- After the 2004–2019 epidemic of chikungunya virus (CHIKV), the largest chikungunya epidemic ever recorded, this disease remains a global problem.
- New treatment options are needed for patients with chikungunya arthropathy, in particular for patients with chronic arthralgia and/or life-threatening manifestations, which primarily present in the very young and the elderly.
- The mechanisms by which CHIKV or viral material persists in joint tissues and drives chronic disease are unclear; characterizing the processes involved might open up new avenues for clinical interventions.
- Better control and evaluation measures are required to prevent transmission of arboviral diseases such as chikungunya.
- The unpredictable nature of chikungunya outbreaks complicates phase III field trials of vaccines; new solutions for trialling these vaccines are needed, which could involve human challenge models and systems vaccinology.

spectrum of disease manifestations, the important antiviral factors and the central mediators of arthritic immunopathology. These insights have led to improved disease classification and management, and have spawned a plethora of potential avenues for new interventions. This Review provides an overview of the lessons learned about chikungunya in the aftermath of the 2004–2019 epidemic. The disease manifestations are outlined, including those associated with acute, atypical acute and severe acute disease, as well as the chronic phase of the disease and its potential sequelae. Disease in infants and children, and mother-to-child transmissions, are also discussed as the clinical presentations in this group of patients differ. Also covered are comorbidities, which increase the risk of severe disease, and co-infections with Zika virus (ZIKV) and dengue virus (DENV).

Disease manifestations of chikungunya

Estimates for the asymptomatic infection rate for CHIKV range from 3% to 82%. The breadth of this range, derived from a comprehensive review of 24 studies, is similar to that found with other infectious diseases and has yet to be fully explained[27]. Four clinical forms of symptomatic chikungunya were proposed in an expert consultation, led by the WHO–Pan American Health Organization: acute, atypical acute, severe acute and chronic (suspected or confirmed)[28,29]. The three acute forms of chikungunya are associated with a range of different symptoms, with confirmation of diagnosis usually achieved by IgM serology (BOX 1; Supplementary Table 3). Other classifications of chikungunya have included a sub-acute phase between acute and chronic[30], with chronic disease defined as disease lasting >3 months[30,31]. Generally, patients with chronic disease do eventually recover (usually within 3–24 months)[18,19], although sequelae might arise. An emerging body of evidence suggest that the IOL lineage is associated with more severe presentations than the Asian genotype[19,32,33]. Infection with other arthritogenic alphaviruses can cause similar acute symptoms (such as fever, polyarthralgia–polyarthritis, rash and myalgia)[1], but rarely result in the atypical or severe manifestations that can occur with chikungunya, although such manifestations have been documented for Mayaro virus infections[34].

Acute chikungunya

The distinctive features of chikungunya onset are usually fever and polyarthralgia, often accompanied by polyarthritis (TABLE 1). The fever is often of rapid onset and high grade, with one large study reporting a mean maximum body temperature of 39.8 °C (SD ±0.5 °C) and fever duration of 4.88 days (SD ±2.7 days)[24]. Polyarthralgia usually starts around the same time as the fever and is often incapacitating, usually symmetrical and primarily involves peripheral joints[19,24,35,36] (FIG. 2). Acute chikungunya commonly also involves a rash, which is usually maculopapular and predominantly located on the trunk and extremities, but also occurs less frequently on the face, palms, or soles[19,37]. The constellation of manifestations typically associated with acute disease (TABLE 1) seems to be considerably less common or less overt in older patients (>65 years of age), who have a much higher frequency of atypical or severe forms of chikungunya (as well as having a higher frequency of comorbidities) than younger patients[29]. Hence (along with inherent variability, different diagnostic criteria and different data acquisition processes), the wide ranges in the percentages of patients with certain symptoms (for example, 10–80% for myalgia) might also reflect the age distribution of patients included in the study cohorts.

Atypical acute chikungunya

A large collection of atypical manifestations of acute chikungunya, affecting a range of systems and organs (for example, neurological, cardiovascular, skin, renal and respiratory manifestations) have been documented, often in hospital settings[10,29,38–40] (TABLE 2). Although most patients with chikungunya admitted to hospital (~80% in one study[10]) do not have severe symptoms, many patients have atypical manifestations that can become severe and/or have chikungunya complicated by co-infections and/or comorbidities. Hospitalization rates for patients with chikungunya have varied from 0.6% (Martinique and Guadeloupe[10]), through 2.3% (Réunion Island[11]), 3.3% (Brazil[12]) and 6% (India[13]) to 13% (Puerto Rico[14]). The mean length of hospital stay reported for these patients was 5 days (SD ±7 days; range 0–146 days) in Réunion Island[11] and 9 days (range 0–46 days) in Martinique and Guadeloupe[10].

Severe acute chikungunya

CHIKV infection can result in severe manifestations, the most prevalent being cardiac or multiple organ failure[17,38,41] (BOX 2). Chikungunya-associated viral sepsis and septic shock can also be fatal; for instance, in one study in Guadeloupe of patients with severe chikungunya, 25 out of 42 patients had septic shock, 12 of whom died[42]. Chikungunya can result in neurological complications[43], with mortality often associated with central nervous system (CNS) diseases including encephalitis and encephalopathy[44]. Renal failure also seems to occur frequently in severe cases of chikungunya[45] and is a reported cause of death[38]. Other rarer causes of chikungunya-related death that have been reported include toxic hepatitis, bullous dermatosis, myocarditis/pericarditis, respiratory failure, pneumonia and acute myocardial infarction[38]. Chikungunya-related

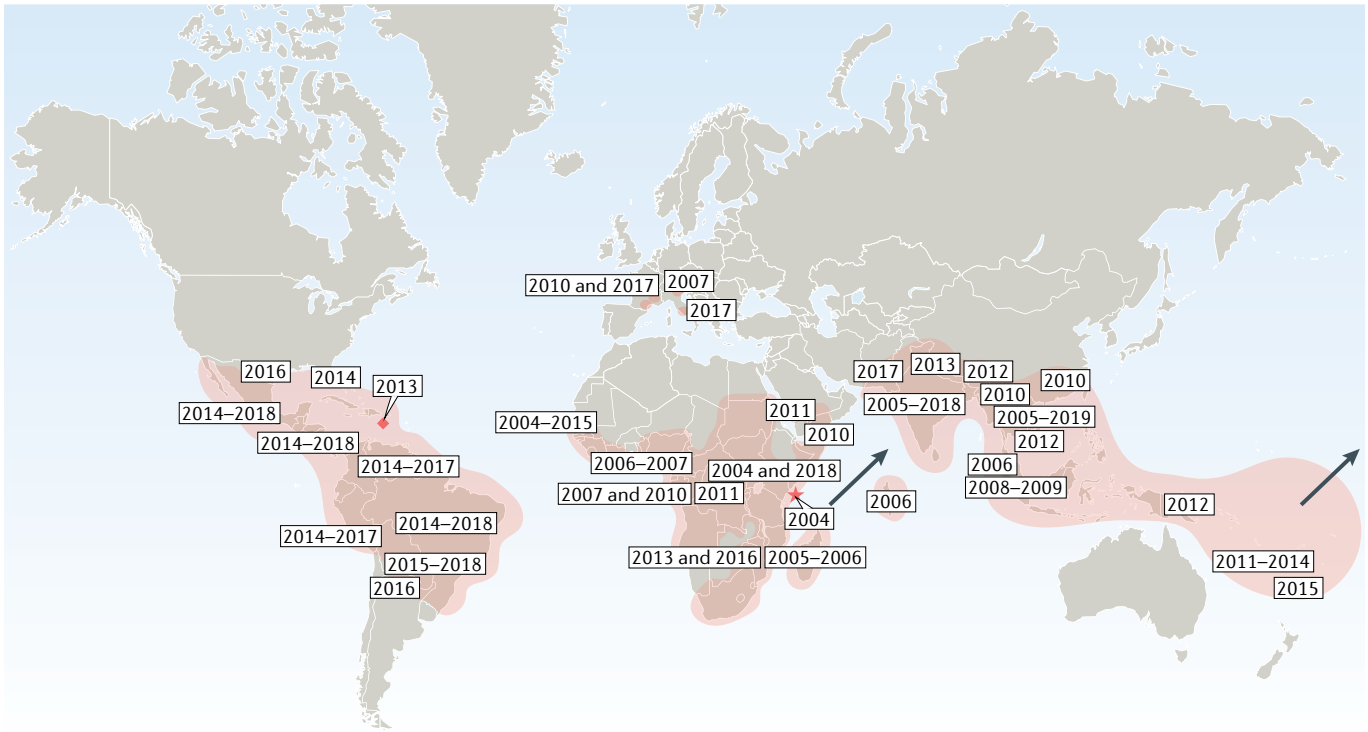

Fig. 1 | **Emergence and spread of the 2004–2019 CHIKV epidemic.** In 2004, the first outbreak of the largest epidemic of chikungunya ever recorded began on Lamu Island, in Kenya (red star). The epidemic then expanded west within Africa, and also spread eastwards across islands in the Indian Ocean to Asia. The epidemic grew within India and Southeast Asia, moved east to the Pacific Islands and reached the Caribbean (Saint Martin Island) in 2013 (red diamond). Saint Martin Island was the first site in the Americas to report autochthonous transmission (completion of a human-to-mosquito-to-human transmission cycle within the same geographical location) of CHIKV. From there, CHIKV spread into Central and South America, and cases were still being reported in 2018/2019. Small outbreaks have also occurred in Europe and southern USA. Three major genotypes are recognized — West African, East–Central South African (ECSA) and Asian — suggesting at least three separate introductions of CHIKV from natural reservoirs into human populations during this epidemic. *Aedes aegypti* was the primary vector in Central and South America, most of Africa and the Pacific Islands. *Aedes albopictus* was the primary vector in Europe and parts of the Indian Ocean, West Africa and Papua New Guinea. Both vectors were likely involved in many parts of Asia.

mortality estimates vary for different regions: for example, studies have reported a mortality of 0.024% in Martinique and Guadeloupe[10]; 0.09% in Brazil[17]; 0.1% in Réunion Island[16]; 0.2% in India[12]; and 0.7% in the Dominican Republic[15]. However, the denominator for these percentages (that is, the total number of individuals with chikungunya in these regions) is often difficult to establish accurately in resource-poor settings. A mortality of 0.1%, derived from Réunion Island, might be viewed as a reliable estimate owing to the developed health care and reporting systems in this overseas region of France. Notably, old age (>40–75 years, depending on the study) is a risk factor for severe disease and mortality for individuals infected with either the Asian or IOL viruses[15,17,30], whereas young age (<1 year) or old age (>65 years) increased the risk of CNS disease in a study on Réunion Island[44].

Chronic disease and sequelae

Arguably the most widespread cause of morbidity in patients with chikungunya is chronic disease, although the percentage, longevity, definition, terminology and evaluation of chronic disease vary widely between studies. A meta-analysis reported that ~25% of patients with chikungunya have had disease for >2 months and ~14% for >18 months[18]. Another meta-analysis of patients with chikungunya (that included patients with non-rheumatological manifestations) suggested that 43% of patients had not recovered within 3 months, and 21% had not recovered within 12 months[19]. However, a prospective study in India of 509 patients with chikungunya reported that all but 0.3% of the patients had recovered within 1 year[46]. The primary symptoms for chronic disease in patients with chikungunya are arthralgia and/or arthritis (up to 79% of patients with chronic disease), alopecia (10–29%) and depression (6–54%)[20,22]. Fatigue, mood disorders and sleep disorders were also common chronic symptoms[19]. Factors predisposing to chronic disease included comorbidities (such as osteoarthritis and diabetes), older age (>35–45 years for joint pain), and high viraemia and severe disease during the acute stage[20]. Chronic arthralgia in chikungunya generally involves the same joints affected during the acute phase (FIG. 2) and the arthropathy is not usually overtly erosive[16,47].

Long-term sequelae of chikungunya include depression, chronic fatigue[22] and other neurological disorders[21]. Difficulties remain in separating true sequelae from the

Viraemia
The presence of virus in the circulating blood.

Box 1 | **Diagnosis of chikungunya**

In 2015, a Pan American Health Organization–WHO expert consultation group provided a definition of a typical confirmed case of chikungunya. This definition can be summarized as "fever and joint pain with acute onset" and either "residing or visiting areas with local transmission of chikungunya" or "laboratory confirmation by immunoglobulin or RT-PCR"[212]. However, the primary symptoms of acute chikungunya (TABLE 1) are often shared with other co-circulating (and occasionally co-infecting) arboviruses such as Zika virus (ZIKV) or dengue virus (DENV), which can complicate the clinical diagnosis[213]. Excluding the possibility of DENV infection in cases of suspected acute chikungunya might be critical, especially for paediatric patients with dengue who might require life-saving intravenous fluids[213]. Some distinguishing features between chikungunya and dengue are provided by the WHO[214] and elsewhere[2,35]. Differential diagnoses for chikungunya include other infectious or autoimmune arthritides, malaria and drug reactions[1]. Commercially available diagnostic test kits for chikungunya are available (Supplementary Table 1); the tests are based on the detection of anti-CHIKV IgM antibodies (serology) or viral RNA (PCR).

progression of underlying comorbidities[19,48], identifying the independent development of new disease entities and/or determining when patients with chikungunya have recovered and returned to the normal community background levels of musculoskeletal disease. The prevalence of musculoskeletal disease is increasing, with disability-adjusted life-years for musculoskeletal conditions having risen by 61.6% between 1990 and 2016, and by 19.6% between 2006 and 2016. Musculoskeletal conditions include >150 diagnoses, with about a third of people worldwide living with a chronic, painful musculoskeletal condition[49]. Therefore, a patient presenting with a musculoskeletal condition might be granted the same diagnostic rigour, regardless of whether or not they had a diagnosis of chikungunya >6–12 months previously.

Disease in infants and children

Both infants and children can develop chikungunya after a mosquito bite, and neonates can be infected via mother-to-child transmission (BOX 3). Infants (<1 year old) with chikungunya are often hospitalized and admitted to an intensive care unit (ICU)[16,50]. The disease usually presents as fever and rash[51,52]; arthralgia is difficult to assess in infants, but is perhaps expressed as irritability and excessive crying[28,50]. Skin rashes are common (~60–80%) and generalized, and include maculopapular rash, pigment changes, vesiculobullous lesions (fluid-filled lesions) and (sometimes extensive) desquamation (skin peeling)[16,50–52]. Atypical symptoms include (sometimes complex) seizures, diarrhoea, tachycardia, viral sepsis and septic shock[51–54].

Acute chikungunya in children (from 1 to 18 years old) is comparable with disease in adults[50,54,55], although the rate of asymptomatic infection might be higher overall than in adults[27,56]. Children (aged 2 months to 12 years) seem to generate stronger innate immune responses than adults[55], which might explain the reduced severity of arthritis and lower rates of chronic arthropathy (5–11%) in children[50,57]. Nevertheless, a study from the 2014 epidemic in the Caribbean reported that 8.7% of children with chikungunya were hospitalized[57]. Common acute atypical manifestations include vomiting and seizures[54]. Severe disease primarily involves the CNS[44,54,58], but chikungunya can also affect multiple systems and lead to severe viral sepsis and septic shock[53,59]. Severe manifestations are occasionally associated with mortality[16,50,54].

Comorbidities and co-infections

Comorbidities

Comorbidities such as hypertension, diabetes (both type I and type II) and cardiac disease can contribute to chikungunya severity and admissions to the ICU[60]. For example, diabetes can increase the severity and duration of chikungunya and in patients with diabetes and hyperglycaemia, chikungunya infection is associated with worsening of diabetic symptoms (such as poor glycaemic control and acute complications)[61]. The presence of comorbidities is also associated with increased morbidity. For example, in a cross-sectional study of a chikungunya outbreak in north-eastern Brazil, 1% of patients with chikungunya had chronic kidney disease (amongst other comorbidities that included diabetes, haematological disorders, liver disease, hypertension and autoimmune diseases); these patients had higher frequencies of the main acute manifestations of chikungunya and higher mortality than patients without chronic kidney disease[12]. In another study of 65 patients with chikungunya who were admitted to ICUs in Martinique and Guadeloupe, 83% had pre-existing underlying comorbidities (hypertension, diabetes, renal disease, cardiac disease or autoimmune disease, including systemic lupus erythematosus) and the mortality rate among these patients was 27%[62]. Similarly, of 64 patients with chikungunya who were admitted to ICUs in French Polynesia, 77% had pre-existing conditions and 28% died[63]. The aforementioned comorbidities also often exacerbate disease after infection with other viruses such as DENV, West Nile virus and influenza virus[60,61]. Perhaps surprisingly, in a case series and literature review of patients with chikungunya undergoing a solid organ transplantation, most patients experienced no graft issues and a benign clinical course of chikungunya, with immunosuppressive treatment perhaps decreasing the risk of severe or chronic chikungunya immunopathological manifestations[64].

Co-infections

The symptoms, vectors and geographic distribution of the arboviruses DENV, ZIKV and CHIKV overlap considerably[3,65,66]. All three viruses are associated with fever, arthropathy and rash, which can complicate clinical diagnoses. All these viruses are also transmitted by *Aedes aegypti* and co-circulate in parts of South America, Africa and Asia, leading to co-infections. For instance, in a cohort of patients with febrile syndrome at the Colombian–Venezuelan border, 7.64% of patients were co-infected with both DENV and CHIKV, and 1.91% were co-infected with DENV, CHIKV and ZIKV[67]. Similarly, in a Nicaraguan study of patients with a suspected arboviral infection, 27% of patients tested positive for two or three of these viruses; however, the presence of DENV and/or ZIKV had no notable effects on CHIKV viraemia[68]. In a study in India, 12.4% of hospitalized patients with acute symptoms of chikungunya had IgM antibodies against both CHIKV and DENV; however, the only disease exacerbation associated with

Arboviruses
Viruses that can be transmitted by arthropod vectors (for example, mosquitoes) to vertebrate hosts (for example, humans)

dual infection was diarrhoea (found in 16.2% of these patients)[69]. Patients with both chikungunya and dengue were also reported to have more severe arthropathy, myalgia, thrombocytopenia and rash than patients with dengue alone[70]. Such dual-infected patients were also more likely to have a rash and be hospitalized than patients with chikungunya alone[71]. The mortality rate is potentially higher in patients infected with both DENV and CHIKV than patients infected with either virus alone, although the evidence is weak given the very low patient numbers[72,73]. Finally, patients with chikungunya and a preceding DENV infection are at a higher risk of developing aggravated chronic chikungunya[74]. However, other studies have reported no notable exacerbation or unique presentations associated with acute dual or triple infections with the aforementioned arboviruses[75,76]. Thus, co-infections do not reliably cause novel clinical manifestations, nor do they generally seem to require unique clinical management[77]. However, patients with a potential DENV infection should not be given aspirin or other NSAIDs until they have been afebrile for ≥48 h and have no warning signs for severe dengue[78].

Co-infections with CHIKV and either HIV[79] or malaria have also been reported[65]. In patients infected with both HIV and CHIKV, lymphopenia was more common, more patients reached the definition of severe immunosuppression, and CD4 counts were lower than in patients infected with HIV alone[79]. Although the effect of CHIKV/malaria co-infections in humans remains unclear, mouse studies suggest that malaria infection can ameliorate chikungunya-related arthropathy[80]. In mice, CHIKV infection can compromise lymph node function[81] and alter CD8 T cell trafficking[82], with such CHIKV-mediated changes potentially modulating adaptive immunity and thus immunopathology in co-infection settings.

Immunopathology

Antiviral versus arthritic responses

In the advent of the recent unprecedented outbreak of chikungunya (FIG. 1), our understanding of the innate and adaptive immune responses induced by CHIKV infection, both in humans and in animal models, has grown substantially[31,83–87]. A range of cells and mediators have been implicated in chikungunya immunopathology (Supplementary Table 4). Importantly, many responses that promote chikungunya immunopathology are also required for protection against viral infections, which is clearly an important consideration in the development and application of new therapeutic interventions.

The type I interferons, primarily IFNβ and subtypes of IFNα, are antiviral cytokines that mediate highly effective protection against alphavirus infection[88]. These cytokines have an important function in limiting the sharp increase in viral replication during the early stages of infection[88,89]. The anti-alphaviral activity of type I interferons is optimal at 37 °C and this activity decreases with decreasing temperatures, being noticeably lower even with a reduction of only 2 °C (REF.[36]). In CHIKV-infected mice, the virus can replicate better in the extremities than elsewhere in the body because these tissues are usually a few degrees cooler, which might explain why the peripheral joints are usually affected in alphaviral arthropathies (FIG. 2)[36]. In the arthritic limbs of mice, up to ~8% of polyadenylated RNA can be of viral origin[90], attesting to the extraordinary replicative capacity of CHIKV in the peripheral joints at slightly reduced temperatures. However, in addition to inhibiting viral replication, type I interferons can also promote arthritis. For example, injection of polyinosinic:polycytidylic acid (a mimic of viral double-strand RNA and potent inducer of type I interferon production) into the feet of mice can induce arthritis, and recapitulates much of the inflammatory gene expression signature that occurs in mouse feet during CHIKV arthropathy[36].

In mice, B cells, T cells and natural killer (NK) cells are not required for survival during an acute infection[91], whereas an intact type I IFN response is critical[88,89]. Deficiencies in components of the complex type I IFN network[88] in the elderly (>65 years)[92,93] and in neonates (<4 weeks old) might explain the increased risk of severe disease in these patient populations. For instance, neonates and very young children (<3 months) have attenuated RIG-I responses (required for the detection and triggering of type I IFN responses)[94] and neonates have impaired interferon regulatory factor (IRF) 7 activation[95] (required for amplification of the type I IFN response[89]). Monocytes from elderly individuals (>65 years old) have reduced expression of TNF receptor-associated factor 3 and IRF8 (both required for optimal RIG-I signalling) compared with monocytes from younger individuals[92]. Elderly individuals can also have slightly lower body temperatures than younger individuals[96,97], which might also result in reduced antiviral type I interferon activity post-infection[36].

The clearance of viraemia requires antiviral antibodies[85,91,98]. Neutralizing anti-CHIKV IgM responses are apparent as early as 4 days after the onset of symptoms[99] and the presence of CHIKV-specific IgG3 antibody responses 7–10 days post-onset of symptoms is associated with more severe acute disease but decreased likelihood of persistent arthralgia[100]. CHIKV-specific CD4 T cells are required for IgG class switching and

IgG class switching
The switching of B cell immunoglobulin production from IgM to IgG antibodies

Table 1 | **Typical symptoms of acute chikungunya**

Symptoms (duration)	Percentage of patients[a]	Refs
Arthralgia (weeks to months)	80–100	24,118,204–206
Arthritis (weeks to months)	62–100	24,204
Fever (usually lasts 1 week)	80–100	1,118,204
Myalgia (usually lasts ~7–10 days)	10–85	1,24,118,205,206
Headache	30–90	24,118,205,206
Rash (usually lasts ~1 week)	36–88	1,24,118,204,205,207
Fatigue	43–67	118,207
Diarrhoea	25	24,205
Oedema	22–39	24,206

[a]Percentages of patients with the indicated symptoms, with the ranges encompassing all referenced studies.

Efferocytosis
The process whereby dying or dead cells are removed by phagocytic cells.

efficient production of anti-CHIKV IgG antibodies[91], but these cells are also major promoters of arthritic inflammation.

Finally, monocytes and macrophages also have antiviral activity against CHIKV[101–103], and are important for efferocytosis[104] and resolution of inflammation[105]. However, as discussed in the next section, these cells are also highly implicated in chikungunya immunopathology.

A major objective for the field has been to identify appropriate pro-inflammatory mediators that can be targeted without compromising protective antiviral responses[86,106]. TNF is induced during CHIKV infections[90,107–109] (FIG. 3), and TNF inhibitors (including etanercept and adalimumab) have shown some promise in the treatment of patients with chikungunya[110]. However, in mice with an active infection of Ross River virus (RRV) (a close relative of CHIKV that causes RRV disease), treatment with etanercept resulted in 100% mortality, indicating that TNF also has important antiviral activities[111]. This protective function of TNF against viral infections might raise concerns about TNF inhibitors for the treatment of patients with chikungunya; however, exacerbation or reactivation of CHIKV infection is unlikely once patients have adequate levels of neutralizing antibodies. Such antibodies (detectable 4 days after the onset of disease) are clearly present in patients with a positive serodiagnosis[99] and have long been present by the time the chronic phase of disease begins[112]. Indeed, in patients with chronic manifestations of chikungunya, treatment with an immune-modulating biologic agent (including infliximab and etanercept) was not associated with overt worsening of disease[113]. An important issue to consider, however, is potentially compromising antiviral immunity in settings where multiple arboviruses are circulating. In such scenarios, therapies that target chikungunya immunopathology should ideally not compromise the patients' ability to generate immunity to subsequent DENV or ZIKV infections.

In addition to the inhibition of antiviral activity, another potential concern of anti-inflammatory interventions is the risk of inadvertently promoting immunopathology. For instance, the chemokine CC-chemokine ligand 2 (CCL2) is strongly induced during CHIKV infections[108,109] and targeting the CC-chemokine

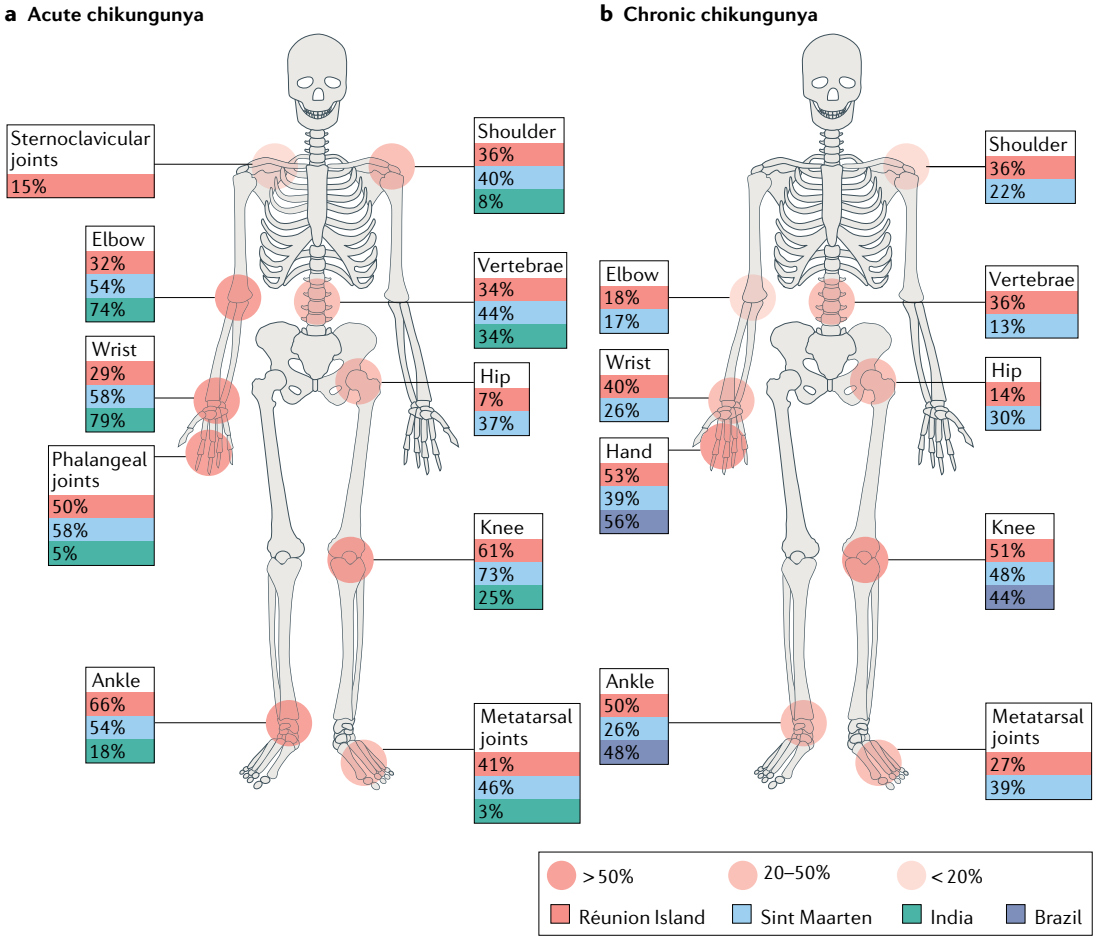

Fig. 2 | **Joints affected by chikungunya arthralgia. a** | Joints with arthralgia at or near the time of disease onset, indicating the range of percentages of patients reporting arthralgia in each indicated joint or group of joints in previous studies of patients with acute chikungunya on Réunion Island[199], on Sint Maarten in the Caribbean[200] or in India[201]. **b** | Joints with arthralgia in patients with chronic chikungunya, based on data from patients with chronic chikungunya on Réunion Island[202], on Sint Maarten in the Caribbean[200] or in Brazil[203]. Assessment methodologies were not standardized in these studies, and so it is difficult to attribute any differences across these studies to CHIKV genotypes or specific populations.

Table 2 | **Atypical symptoms of acute chikungunya**

Systems/organs affected	Percentage of hospitalized patients[10]	Manifestation examples	Refs
Neurological	40	Encephalitis Meningoencephalitis Guillain–Barre syndrome	38–41,43
Cardiovascular	27	Hypotension Myocarditis Arrhythmias	38–41
Skin	10	Hyperpigmentation Bullous dermatosis Erythema	38–41
Renal	26	Albuminuria Haematuria Nephritis	39,40,45
Respiratory	14–26	Dyspnoea Respiratory failure Pneumonia	38,39,41
Vascular	10	Haemorrhagic signs Bleeding gums Melena	39,41,205,208,209
Ocular	Less common than other atypical symptoms	Conjunctivitis Photophobia Retinitis	39,41,210,211
Liver	Less common than other atypical symptoms	Hepatitis Hepatomegaly Altered function	38,40,41

Atypical acute manifestations can accompany the typical acute symptoms (TABLE 1). Atypical manifestations are grouped by the systems/organs affected, with some examples of manifestations provided; these are neither complete nor ranked and the reader is directed to the accompanying references for a full description of manifestations.

receptor 2 (CCR2)–CCL2 axis in mice reduces the recruitment of inflammatory monocytes and macrophages into the joints[104]. However, in the absence of monocytes and macrophages, neutrophils are instead recruited into the joints of CCR2$^{-/-}$ mice post-CHIKV infection, promoting joint destruction[104].

Mechanisms of immunopathology

Taking synovial biopsies or aspirates from patients with alphavirus-induced arthritis is often difficult to justify, as such procedures carry a small risk for the patient and usually have a negligible effect on disease management. Nevertheless, a small number of studies have analysed such material from patients with chikungunya or RRV disease[112,114–116]. As with other viral and bacterial arthritides[117], CHIKV-related and RRV-related arthropathies probably arise from innate and adaptive immune responses stimulated by viral material in joint tissues[1,90,91,109,118] (FIG. 3).

The ability of CHIKV to affect multiple systems/organs might be because of the virus's predilection for infecting fibroblasts[47,119], a cell type that is present in many tissues and organs (including the connective tissue, skin, synovium and periosteum[89,119]). The widespread expression of the arthritogenic alphavirus receptor, matrix remodelling-associated protein 8 (MXRA8), also probably contributes as this receptor permits infection of a large range of different cell types[120]. These cell types include circulating monocytes[108], macrophages[109], endothelial cells[89,109], cells of the nervous system[43,91] and skeletal muscle cells[47,89], as well as cell types present in joints (FIG. 3). Infection usually induces cell death[121], mainly by apoptosis,[104,106] but also to a lesser extent by necroptosis and pyroptosis[122]. Cell death might directly contribute to pathology, especially for neurological manifestations[91,122,123]. However, immunopathology probably has the major role in the majority of rheumatic manifestations (FIG. 3, Supplementary Table 4).

Macrophages and monocytes. Arthritic infiltrates in patients with alphavirus-associated arthritides predominantly comprise mononuclear cells, mostly consisting of monocytes and macrophages but also including T cells, B cells and NK cells. In contrast to autoimmune arthritides, neutrophils are uncommon in the synovial infiltrates of patients with alphaviral arthritides[101,112,114–116,124]. Monocytes and macrophages are strongly implicated in chikungunya arthritic immunopathology

Synovial macrophages from patients with chikungunya have an activated morphology, with a ballooned appearance and multiple vacuoles[112,116], indicative of a phagocytic (activated) phenotype[108,125]. Cytokines induced during CHIKV infection, such as type I interferons, IFNγ and TNF, are well-known activators of monocytes and macrophages (FIG. 3). Studies in non-human primates suggest that macrophages are the likely site of the persistence of CHIKV and CHIKV material[109]. Alphaviral RNA and/or proteins have also been detected in the synovial macrophages of patients with chikungunya and RRV disease[112,114]. In vitro work in RAW264 cells (a murine macrophage cell line) suggests that CHIKV-infected macrophages are a source of arthritogenic cytokines such as TNF and IL-6[126]. Mouse models of chikungunya arthritis and analysis of peripheral blood mononuclear cells from patients have also suggested a function for the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome (and thus IL-1β and IL-18) in chikungunya, with a small-molecule inhibitor of NLRP3 activation able to reduce CHIKV-induced inflammation in mice[127]. Notably, NLRP3 is also implicated in the pathogenesis of rheumatoid arthritis (RA), with high levels of NLRP3 activation being reported in monocytes/macrophages infiltrating the synovia of patients with RA[128].

As well as synovial macrophages, whole-blood RNA transcriptomic analyses in paediatric patients suggest that CHIKV infects peripheral blood monocytes (and dendritic cells) and that CHIKV induces a monocyte-centric pro-inflammatory response[108]. CCL2 is a major product of CHIKV-infected monocytes[129], is strongly induced during CHIKV infection[101,109] and is important for the recruitment of monocyte and macrophages into the inflamed joint[104]. CHIKV- infected monocytes also produce other pro-inflammatory mediators, including IFNα, IL-12 and CXC-chemokine ligand 10 (CXCL10)[130] (FIG. 3).

Overall, monocytes and macrophages have a very large number of functions and differentiation states[131] and interact with CHIKV on a range of levels; not only are these cells the sites of infection and persistence and the source of pro-inflammatory cytokines, these cells also have antiviral activity and are required for the resolution of inflammation.

CD4 T cells. CHIKV-specific CD4 T cells have been repeatedly implicated as important promoters of CHIKV-mediated arthritis[80,132–136]. Furthermore, regulatory T cells can ameliorate chikungunya arthropathy in mice[137] and are also associated with disease resolution in humans[134]. In mouse models of chikungunya, CD4 T cells infiltrated into the joints in a CXC-chemokine receptor 3 (CXCR3)-dependent fashion[80], with arthritogenic CD4 T cells seeming to have a dominant type 1 T helper (T_H1) cell phenotype[135], expressing the transcription factor T-bet[104] and IFNγ[32,90,101]. Notably, IFNγ-expressing cells are also present in synovial biopsy samples from patients with RRV disease[114]. Curiously, IFNγ deficiency has no major effects on mouse models of chikungunya[90,138]. IFNγ expression was also not detectable in the synovial fluid of one patient with chronic chikungunya, although IFNγ was abundant in their blood during the acute disease phase[112]. The contribution of IFNγ in chikungunya thus remains unclear, although it should be noted that IFNγ is reported to have a complex and pleiotropic function in RA[139,140].

More studies are required to better understand the mechanisms whereby CHIKV-specific CD4 T cells drive arthropathy. Conceivably, rather than being reliant on IFNγ, CHIKV-specific T_H1 cells could activate monocytes and macrophages via interactions involving CD28 and CD80–CD86[136,141], resulting in TNF and IL-6 production[142]. Alternatively, instead of IFNγ expression by T_H1 cells, TNF expression by CD4 cells might have an important function in promoting chikungunya arthropathy[135]. TNF-expressing CD4 cells have a potential pathogenic function in psoriatic arthritis[143] and are targeted by methotrexate in RA[144,145]; notably, methotrexate has shown some benefit in treating chikungunya[146,147].

Some animal models[104,109,148] have suggested the involvement of IL-17 and T_H17 cells in alphaviral arthritides. Concentrations of IL-17 are marginally increased in the plasma of patients with chikungunya during the acute phase of disease compared with that of uninfected individuals[149] and remain increased during the chronic phase[150]. By contrast, IL-17 is not increased in the blood of young patients with chikungunya[108], who are known to experience less severe arthropathy than older patients. IL-17 is implicated in cartilage destruction and bone erosion in RA[151], whereas radiographically detectable joint damage is not generally a feature of alphaviral arthropathy. Nevertheless, some patients with RRV disease have an increased receptor activator of nuclear factor kappa-B ligand (RANKL) to osteoprotegerin (OPG) ratio, indicative of increased osteoclastogenesis and bone resorption[148]. The levels of matrix metalloproteinase 2 (MMP2) messenger RNA were increased in the synovial fluid of one patient with chronic chikungunya (compared with levels in healthy individuals)[112]. Furthermore, concentrations of connective tissue metabolites (proline, hydroxyproline and mucopolysaccharides) were increased in the urine of patients with chikungunya during the first week post-onset of fever[152]. Thus, although alphaviral arthritides might be associated with some IL-17 production, cartilage destruction and bone erosion, the contribution of these processes to alphaviral rheumatic pathology seems to be substantially less important than their role in RA.

NK cells and NK T cells. Synovial NK cells (characterized by their $CD56^+$ $CD3^-$ expression) in patients with chronic chikungunya express the activation marker CD69[112], and data from mouse models suggest that NK cells have a pathogenic role in acute arthropathy[32,90,91]. NK cells from the peripheral blood of patients with acute chikungunya have an activated profile (including the expression of the heterodimer CD94:NKG2C) and are strongly cytotoxic, but secrete minimal levels of IFNγ[153]. NK cells in the peripheral blood of patients with chronic chikungunya express reduced levels of perforin, but they do not express notably higher levels of TNF or IFNγ compared with NK cells from healthy individuals[107]. Increased numbers of synovial $CD56^+$ NK cells in patients with established RA probably promotes arthritis via secretion of TNF and IFNγ[154]; however, the mechanisms by which NK cells contribute to chikungunya arthropathy remain to be elucidated[90].

In addition to NK cells, natural killer T (NKT) cells (characterized by their $CD56^+$ and $CD3^+$ expression) that express TNF or IFNγ are increased in the peripheral blood of patients with chronic chikungunya compared with healthy individuals, and are similarly increased in patients with RA compared with healthy individuals[107]. Hence, NKT cells are likely to contribute to both chikungunya and RA arthropathy, but the underlying mechanisms and their importance remain unclear.

Non-haematopoietic cells. Fibroblasts in connective tissues are a major target of CHIKV infection and produce various IFNα subtypes and IFNβ[119], cytokines with well-described arthritogenic properties[36]. In vitro, CHIKV-infected human synovial fibroblasts secrete RANKL, IL-6, IL-8 and CCL2, and supernatants from these cultures can stimulate osteoclastogenesis[155,156].

Box 2 | Severe symptoms of acute chikungunya

Severe symptoms of acute chikungunya (listed below) are defined as manifestations that include dysfunctions of at least one organ or system that threatens life and requires hospitalization. The term "failure" reflects a spectrum that includes non-lethal manifestations with recovery.

- Cardiac failure[17,38,41,42]
- Multiple organ failure[17,38]
- Viral sepsis and/or septic shock[42]
- Renal failure[10,17,38,42,45]
- Liver failure[10,17,38,42]
- Respiratory failure[10,17,38,42]
- Encephalitis or meningoencephalitis[17,38,42,43]
- Bullous dermatosis[17,38]

Box 3 | **Mother-to-child infections**

Mother-to-child transmission usually occurs during birth and occurs in about half of viraemic (and symptomatic) mothers[215,216]. Of the infected neonates, ~50% develop disease within 3–7 days and 2.8% of cases result in fatality[215]. In a study of mother-to-child transmissions in Réunion Island, 21% of the infected neonates had persisting disabilities[217]. Symptoms vary widely and include fever, poor feeding, irritability (hyperalgesia), respiratory distress, diffuse limb oedema, rashes, sepsis-like illness, meningoencephalitis and other central nervous system abnormalities, and haemorrhagic and cardiac manifestations[215,218–220]. Neurodevelopmental delays can also occur, with one study reporting that >50% of previously symptomatic neonates had a global neurodevelopmental delay at 2 years compared with 15% of uninfected children[220].

A clinical trial was planned in 2014 to evaluate treatment of infected neonates with hyperimmune human anti-CHIKV immunoglobulins to suppress infection, although no results have been posted to date[221]. Another potential treatment, yet to be formally tested, is the use of tocolytic drugs to delay delivery of the baby by a few days, thereby allowing maternal antibodies to resolve the viraemia prior to the onset of labour[215,217]. Tocolytic therapy is used to delay or prevent pre-term delivery[222], and is generally not recommended after 34 weeks of gestation[223]. Nevertheless, encouraging data have been reported for the use of tocolytics to delay birth in mothers with a DENV infection[224], although further studies are required to establish the safety and efficacy of this approach in chikungunya.

Zika virus (ZIKV) recently caused international concern owing to its association with congenital Zika syndrome (CZS), which describes a pattern of birth defects (including microcephaly) caused by infection during pregnancy. The largest outbreak of CZS occurred in Brazil (2014–2016) and the postulated reasons for the unprecedented outbreak have been varied and remain controversial. The results of one epidemiological analysis in 2018 led the investigators to speculate that CHIKV infection might somehow increase ZIKV severity[66]. CHIKV infections are not usually associated with congenital abnormalities, although the development of microcephaly after birth in CHIKV-infected neonates has been reported[220,225]. Rare cases of placental and transplacental CHIKV infection have also been reported, and resulted in abortion[226].

CHIKV can infect human osteoblasts in vitro, which promotes IL-6 and RANKL secretion by these cells and inhibits OPG secretion (increasing the RANKL to OPG ratio)[157]. Notably, the RANKL to OPG ratio is increased in the synovial fluid of patients with RRV disease compared with synovial fluid from healthy controls, and treatment with an anti-IL-6 antibody can reduce bone loss in a mouse model of RRV infection[158]. In addition to osteoblasts, CHIKV can also infect human chondrocytes in vitro[120] and mouse chondrocytes in vivo[89,159]. RRV infection of chondrocytes induces the secretion of IL-6, CCL2, IFNγ and TNF[160]. Thus, multiple non-haematopoietic cell types in the joint can be infected with CHIKV and might contribute to the pro-inflammatory milieu (FIG. 3).

Human skeletal muscle cells (in vitro)[120] and mouse skeletal muscle cells can also be infected by CHIKV in vivo[89], although some evidence suggests that, in humans, only skeletal muscle progenitor (satellite) cells are infected[161]. Infection of skeletal muscle might be responsible for myalgia in patients with chikungunya (TABLE 1); notably, studies of mouse models of chikungunya have reported pronounced inflammatory infiltrates in skeletal muscle tissues[101,111].

Chronic arthropathy

The underlying inflammatory stimuli responsible for chronic chikungunya arthropathy remain unclear[20,47]. The persistence of the virus or viral material[112,114,117], as well as host cell debris[162], in joint tissues probably have important contributions. RNA-seq transcriptional profiling data from mice suggest that chronic inflammation in chikungunya is simply a prolongation of the acute inflammatory response[90], which continues until the viral material is cleared[91]. The expression of a number of pro-inflammatory cytokines and chemokines in the peripheral blood are associated with chronic disease in patients with chikungunya[46,83,118] (Supplementary Table 4). Notably, the expression of pro-inflammatory mediators IL-6, IL-8, CCL2 and IFNα were upregulated in the synovial fluid of a patient with chronic chikungunya compared with expression in serum from the same patient[112].

In chikungunya, the virus and/or viral material seem primarily to persist in monocytes and macrophages in the joints[109,112], which is a feature common to a number of arthritogenic viruses and bacteria in humans and animals[117,163]. Virus-induced apoptosis and reinfection of cells via apoptotic blebs is one postulated mechanism by which alphaviral infection might persist in vivo in the presence of neutralizing antibodies[125,164]. However, although viral material (RNA and/or protein) can be detected, researchers have been unable to isolate infectious (replication competent) virus from the joint tissues of patients with subacute or chronic disease[112,114,116] or from mice 2 weeks after CHIKV infection[91]. A number of possibilities could explain this apparent discrepancy: levels of infectious virus might simply be too low to be isolated; defective viral RNA could continue to replicate as replicons without being able to produce infectious (replication-competent) virus[165], with viral proteins being translated from replicon RNA, and/or residual inactive viral material is only slowly cleared[91], with large amounts accumulating during the acute infection[90].

Although CHIKV arthropathy shares many features with RA[37,83,118,132], there is no clear evidence that autoimmune disease is involved or induced. However, viral material could not be found in synovial fluid from 38 patients with arthropathy at 22 months post-onset of chikungunya, perhaps arguing that arthropathy at this stage is no longer attributable to viral persistence and that alternative mechanisms are in play[166]. The investigators suggested that (as yet undefined) autoimmune sequelae might be responsible (although levels of cytokines or chemokines were not assessed in this study)[166].

Arthralgia is a dominant feature of acute chikungunya and the main symptom of chronic chikungunya (FIG. 2). Alphaviral arthralgia probably involves inflammatory pain, perhaps driven by inflammatory cytokines; however, the mechanisms involved in both inflammatory pain in general[167] and in alphaviral arthralgia specifically remain poorly understood. One might speculate that IL-6 has a role in driving alphaviral arthralgia, as this cytokine features prominently in acute and chronic chikungunya[46,112] (FIG. 3) and anti-IL-6 drugs seem effective in treating pain and fatigue in RA[168]. Chikungunya arthralgia can also have neuropathic characteristics[169,170], which might involve infection and disruption of cells of the peripheral nervous system[21,43,91]. Further research is needed to unravel the mechanisms that underpin

Replicons
Viral RNAs that can self-replicate as they encode genes required for viral RNA replication (including RNA-dependent RNA polymerase), but that are unable to form an infectious virus because of defects in, or loss of, one or more structural genes required for virus particle assembly.

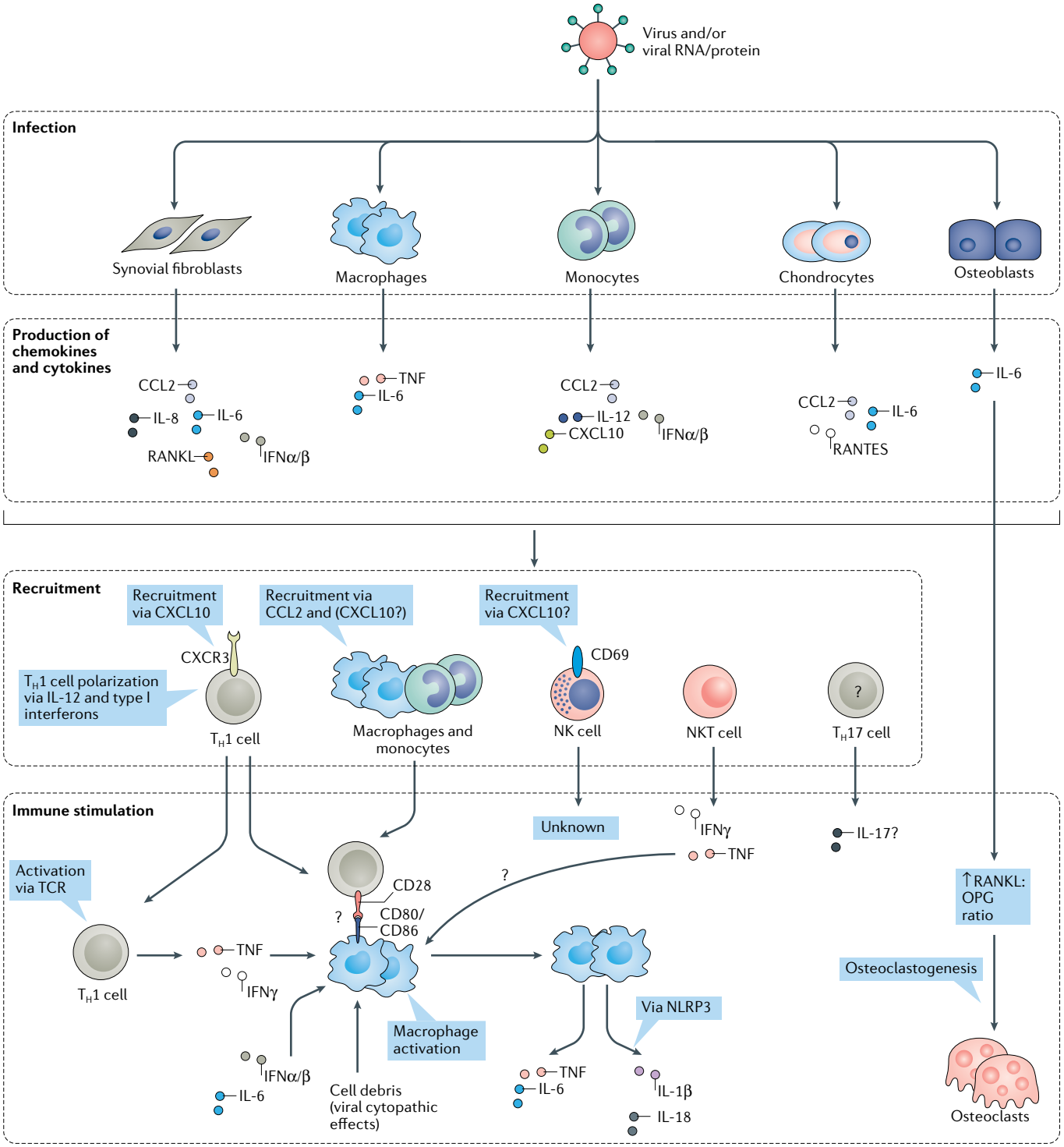

Fig. 3 | **Potential mechanisms of arthritic immunopathology in chikungunya.** Cells and mediators thought to be involved in chikungunya arthropathy (derived from in vivo, in vitro, animal and human studies). Chondrocyte involvement is based on Ross River virus studies. CHIKV probably infects a range of cell types in the joints, resulting in the secretion of multiple pro-inflammatory mediators. Type I interferons (IFNα and IFNβ) are produced, but antiviral activity is probably compromised by the lower temperatures in peripheral joints. Type 1 T helper (T_H1) cell recruitment probably involves CXC-chemokine receptor 3 (CXCR3; a receptor for CXC-chemokine ligand 10 (CXCL10)), and IL-12 and type 1 interferons are known to drive T_H1 cell polarization (activation of CD4 T cells such as T_H1 cells usually occurs via T cell receptor (TCR) engagement). CC-chemokine ligand 2 (CCL2) and CXCL10 recruit monocytes and macrophages, and CXCL10 can also recruit natural killer (NK) cells. NK cells express the activation marker CD69 and probably contribute to arthropathy, but the mechanisms involved remain unclear. Macrophages are activated by a number of cytokines and might also be activated via CD28 and CD80/86. T_H1 cells drive arthropathy, although their secretion of IFNγ is largely dispensable for this activity. IL-17 and T_H17 cells might have a role, although neutrophils are usually absent. Osteoblast and chondrocyte infection and osteoclastogenesis might result in some joint damage, although this joint damage is not generally radiologically detectable. NKT cell, natural killer T cell; NLRP3, NOD-, LRR- and pyrin domain-containing 3; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κ-B ligand; RANTES, regulated on activation, normal T cell expressed and secreted.

Box 4 | **Mosquito control measures**

Non-human primates are believed to be the major reservoir for chikungunya virus (CHIKV), and the virus is transferred via mosquito vectors (mainly the forest or savannah *Aedes* species). Occasional introduction of the virus into urban areas initiates sporadic outbreaks that involve human-to-mosquito-to-human transmission cycles[40]. *Aedes aegypti* was traditionally the primary vector, and remains an important vector for CHIKV. However, in the 2004–2019 epidemic, Indian Ocean Lineage isolates emerged that, compared with other strains, had an increased capacity to be transmitted by *Aedes albopictus* owing to a mutation in the surface glycoprotein (E2, A226V) of CHIKV (although other mutations might also be involved)[227]. *Aedes albopictus* is an aggressive biter with an ever-increasing global distribution[228] and tends to bite humans outdoors during the day or early evening; thus, CHIKV transmission is minimally affected by bed nets[229]. Air-conditioned premises tend to have low mosquito numbers (as windows are usually closed), but the expense of this equipment precludes its widespread use in poor communities[230]. Use of mosquito repellent is often limited, even after media campaigns and distribution of free repellent[230]. Use of insecticides in public health settings remains the cornerstone of control efforts; however, whether this approach is actually effective against *Aedes* and associated arboviral diseases is unclear[231]. The global growth of insecticide resistance also further compromises control measures[229]. Alternative control strategies are being developed, such as introducing an insect toxin into a fungus that infects mosquitoes[232]. Another advanced example uses *Wolbachia* (a Gram-negative bacteria that infects mosquitoes), with a large field trial of *Wolbachia*-infected *A. aegypti* currently underway in Yogyakarta, Indonesia, with the hope that transmission of DENV, ZIKV and CHIKV can be reduced[233]. The approach has already had promising results in reducing cases of dengue in Townsville, Australia[234].

alphaviral arthralgia, with such endeavours hopefully leading to new therapeutic approaches.

Treatments and vaccines

Anti-inflammatory therapy

A number of consensus guidelines[30,146,171], reviews[2,35,113,172–174] and perspectives[37,175] for the treatment and management of chikungunya are available. In summary, acetaminophen (paracetamol) is recommended for the initial treatment of fever and pain. If pain-relief is inadequate, NSAIDs are the mainstay of treatment (except in patients with a suspected DENV infection[78]). However, NSAIDs are contraindicated in several comorbidities, including uncontrolled hypertension, kidney disease and inflammatory bowel disease, and NSAIDs should be discontinued in pregnant patients 6–8 weeks before birth. Low-dose corticosteroids (with or without NSAIDs) seem to be effective in NSAID-refractory patients[35,113,146,171,175], although the potential adverse effects of these drugs should be considered in risk–benefit assessments[31,176]. For patients with chronic chikungunya who are refractory to the aforementioned treatments, DMARDs have shown some efficacy[113,172], and sulfasalazine and methotrexate have been suggested as first-line options[146,147]. However, chikungunya arthropathy is usually not overtly erosive[16,47], and so DMARDs might seem hard to justify[177] unless an underlying destructive autoimmune disease is present. Methotrexate treatment, in particular, can have rare but potentially serious adverse effects and requires extensive clinical monitoring. Another DMARD, chloroquine, has also been reported to worsen disease[178].

Targeting pathogenic CD4 T cells has shown some promise in animal models of chikungunya. For example, treatment with abatacept, a CTLA4-Ig fusion protein that interferes with T cell activation, ameliorated chikungunya in mice without affecting viraemia; however, this therapy was only partially effective unless combined with an antiviral antibody[136]. Although biologic drugs are an exciting new avenue for targeting specific arthritic pathways, the high cost of these drugs might preclude their widespread use, especially in resource-poor settings. Human data for the use of biologics in the treatment of chikungunya are also currently limited, inconclusive and/or complicated by autoimmune comorbidities[113,172]. Finally, fingolimod (a sphingosine 1-phosphate receptor agonist that is used to treat relapsing forms of multiple sclerosis[179]) has also shown preliminary potential for treating chikungunya. Treatment with this agonist, which sequesters lymphocytes in lymph nodes to prevent their participation in tissue inflammation, was able to abrogate chikungunya in a mouse model[133]; however, the cost of this drug might limit enthusiasm for this treatment in humans, especially in resource-poor settings.

Antiviral treatments

There has been substantial preclinical evaluation of antiviral chemotherapeutic drugs for inhibiting CHIKV infection, which will not be reviewed herein as few of these drugs have been tested in vivo and none has reached or shown efficacy in human clinical trials. Whether an antiviral approach seeking to inhibit viral replication would be effective against CHIK is unclear. In most patients, by the time a diagnosis has been reached and treatment has been initiated, virus and/or viral RNA replication could be largely over. Conceivably, low-level RNA replication (evidence of which is currently lacking in patients) might drive chronic disease and could thus be targeted by antiviral drugs.

Antiviral monoclonal antibody treatments have also shown promise in mouse models[103,180,181]. However, by the time a serodiagnosis of chikungunya is obtained (BOX 1), patients usually already have antiviral antibodies, and chronic disease occurs despite ongoing robust antibody responses[112]. The settings and window of opportunity wherein such antibody treatments might be effective might thus be quite limited[133], and the high cost of such antibodies will probably limit their widespread use.

Vaccine development

As well as treatment strategies, disease prevention measures (such as vaccines and mosquito control measures (BOX 4)) are in development. CHIKV vaccines most often use the structural polyprotein of CHIKV; this polyprotein is cleaved into five proteins (E1 and E2 viral spike glycoproteins, capsid, E3 and 6 K) that assemble into a viral particle, thereby presenting an authentically folded quaternary structure to the immune system[182,183]. Vaccination seeks to recapitulate naturally acquired protective immunity (generated after infection with CHIKV) and induce neutralizing antibodies directed at the viral spike glycoproteins (comprising E1/E2 trimers). Such antibodies are thought to be the main mediators of protection[98] by blocking the virus from binding to the receptor[120], by blocking viral entry into cells and/or by preventing viral budding[180,184].

Virus-like-particle vaccine
A protein-based vaccine that recapitulates the appearance and structure of a virus particle, but that has no capacity to replicate in the vaccine recipient because, for instance, the viral genome is (in part or wholly) missing.

Human challenge model
In a CHIKV vaccine context, volunteers are vaccinated with a CHIKV vaccine and are then infected with CHIKV (likely an attenuated CHIKV for safety reasons) in a controlled hospital setting (distinct from conventional phase III trials where vaccine recipients are released into the community and can acquire CHIKV naturally).

Systems vaccinology
A systems-based approach in which transcriptional profiling (followed by bioinformatic analyses) is used to obtain a detailed picture of changes in gene expression following vaccination.

Systems serology
A systems-based approach that measures biophysical and functional characteristics of antigen-specific antibody responses (for example, responses to vaccination); measured characteristics include immunoglobulin isotypes, Fc receptor binding profiles, antibody glycosylation patterns and antibody affinity.

Serogroup
For viruses, a serogroup means that viral infection with one member of that serogroup will generate antibodies capable of recognizing (cross-reacting with) other members of that serogroup.

The global market value for a CHIKV vaccine has been estimated to be ~€500 million (~US$ 600 million) annually; however, this value might be viewed as relatively low compared with other projects (for instance, the global influenza vaccine market value in 2018 was estimated to reach >US$ 5 billion)[185]. In a workshop in India in 2018, the Coalition for Epidemic Preparedness Innovations (CEPI) reported that four vaccines are in phase I human clinical trials and two vaccines are in phase II clinical trials[186]; the latter being a recombinant measles virus vaccine that encodes the CHIKV structural polyprotein[187] and a virus-like-particle vaccine[188]. Unfortunately, phase III field trials for epidemic diseases such as CHIKV are complicated by the inability to predict the geographical location and size of the next outbreak[189]. An alternative or complementary approach to phase III field trials might involve a human challenge model (as described for dengue[190]), in which, for instance, vaccine recipients might be challenged with a live attenuated CHIKV[191]. Such studies might be combined with systems vaccinology and systems serology approaches, which should help to provide more sophisticated correlates of protection[192,193].

All CHIKV genotypes seem to belong to a single serogroup[194,195]. However, variations in cross-neutralization capacities have been reported, such that antibodies raised to one CHIKV genotype are relatively less efficient at neutralizing a different CHIKV genotype[33,196]. Vaccines currently in development use CHIKV antigens from one CHIKV genotype; whether such (single valent) vaccines will provide broadly comparable protection against all CHIKV genotypes thus remains to be established. Another challenge will be timely deployment in rapidly evolving outbreaks; for example, during the Réunion Island outbreak, the number of infected individuals began to escalate at the beginning of 2006, but the epidemic was largely over by July 2006[197].

Conclusions

The unprecedented 2004–2019 CHIKV epidemic has resulted in a surge of research into chikungunya, and has led to many new insights and consensus guidelines for clinical management. There is a clear need for better treatment options for patients with chikungunya and NSAID-refractory arthropathy, chronic arthralgia or severe, life-threatening disease. Well underway are preclinical and clinical investigations of new drugs, and drugs developed for other inflammatory arthritides (such as RA), for treating chikungunya arthropathy. Such endeavours should also facilitate treatment of arthritic disease caused by other alphaviruses such as Mayaro virus and RRV, which have the potential to cause alphavirus outbreaks[34,198]. However, expensive treatments are unlikely to be widely adopted in resource-poor communities and in high-attack-rate settings. Protracted chronic chikungunya (particularly chronic arthralgia) remains poorly understood and complicated by comorbidities and high background levels of musculoskeletal pain in the community. Mosquito control measures are hampered by insecticide resistance and the difficulties in judging whether interventions actually effect disease prevalence. Some vaccines are in advanced stages of development; however, the limited market size does not provide a clear financial incentive for the development of vaccines, and outbreaks are unpredictable. New agencies (such as the CEPI) and technologies are probably needed to bring such interventions to the market[193].

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Competing interests
A.S. declares that he is a consultant for Sementis Ltd., a company that is developing vaccines against chikungunya virus and Zika virus. A.S. declares that he has been a consultant for Valneva and GSK, which are also developing CHIKV vaccines.

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The IL-1 family of cytokines and receptors in rheumatic diseases

Charles Anthony Dinarello[1,2]

Abstract | More than any other cytokine family, the 11 members of the IL-1 family are associated with innate immune responses, which occur in acute inflammation and chronic inflammatory conditions such as rheumatic diseases. In many rheumatic diseases, the severity of the condition can result from the balance between the pro-inflammatory and anti-inflammatory members of the IL-1 family. Pro-inflammatory family members (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β and IL-36γ) are found in the articular environment during arthritis and often correlate with the degree of inflammation present. IL-1β has emerged as pivotal for promoting inflammation, particularly in autoinflammatory diseases, whereas IL-1α and the IL-36 subfamily are associated with skin diseases. IL-33 regulates T helper 2 (T_H2) cell-mediated diseases, in sharp contrast to IL-18, which mainly regulates T_H1 cell-mediated responses. The IL-1 family also contains four members that suppress inflammation: two specific receptor antagonists (IL-1 receptor antagonist (IL-1Ra) and IL-36 receptor antagonist (IL-36Ra)), and two members that broadly suppress innate inflammation by non-specifically reducing several cytokines and chemokines (IL-37 and IL-38). In this Review, each of the eleven IL-1 family cytokines and their receptors are discussed, along with their putative roles in rheumatic disease and therapeutic options for targeting or promoting these cytokines.

The history of the IL-1 family of 11 cytokines begins with the discovery of the first two members, IL-1α and IL-1β. These family members were described in 1974 as two molecularly distinct 'leukocytic pyrogens'[1]. As the name pyrogen suggests, these proteins promote fever. In 1977, one of the two human leukocytic pyrogens was purified to homogeneity and had the unusually high specific activity of producing fever at a dose of 10 ng/kg (REF.[2]); however, it was not until the cDNA of this human leukocytic pyrogen was cloned in 1984 (REF.[3]) that its name was changed to IL-1β. The pro-inflammatory nature of IL-1β was confirmed by the ability of a recombinant form of human IL-1β to produce fever in humans at a dose of 10 ng/kg (REF.[4]). Also in 1984, a macrophage supernatant product that augmented T cell function, termed 'lymphocyte activating factor' was cloned in mice; cDNA analysis revealed that there was a second gene coding for IL-1, which was named *IL1A*[5]. Human IL-1α was cloned in 1985 and recombinant human IL-1α also produced fever in humans at a dose of 10 ng/kg (REF.[4]). Before the availability of recombinant IL-1α or IL-1β, the IL-1 family had a role in rheumatology. In 1977, 'mononuclear factor', a supernatant from activated human monocytes similar to the supernatants containing leukocytic pyrogen or lymphocyte activating factor, induced the production of prostaglandin E2 (PGE_2) by human synovial cells in vitro[6]. A few years later, another study showed that 'lymphocyte activating factor' could induce collagenase production by human synovial cells in vitro[7]. Another milestone study in the emerging role of IL-1 family cytokines in rheumatology came in 1980, and showed the ability of 'catabolin', a protein produced by synoviocytes, to break down cartilage[8]. Similar to leukocytic pyrogen, mononuclear factor, lymphocyte activating factor and catabolin were each later recognized to be either IL-1β or IL-1α.

In 1981, naturally occurring IL-1 inhibitory activity was first noted to occur in the circulation of humans during endotoxaemia[9]. A specific inhibitor of IL-1 activity was subsequently isolated from human monocytes[10] and from the urine of children with juvenile arthritis[11]. In 1987, this IL-1 inhibitor was identified to be a receptor antagonist for IL-1α and IL-1β[12]. This milestone observation catapulted the IL-1 family into clinical rheumatology research, and blocking the IL-1 receptor with a natural inhibitor paved the way for IL-1-blocking therapies. The cDNA for the IL-1 receptor antagonist (IL-1Ra), the third family member to be discovered, was reported in 1990 (REF.[13]), and soon after, a recombinant form was developed into the biologic therapy anakinra, which is now used to treat a broad spectrum of rheumatic diseases[14] (Supplementary Table S1).

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Key points

- The IL-1 family of cytokines contains 11 members that either promote inflammation or specifically or non-specifically limit inflammation.
- The main functions of the IL-1 family are innate immune reactions and inflammation, rather than acquired immunity.
- IL-1β has emerged as an important cytokine in the pathogenesis of several rheumatic diseases, and can be targeted to treat these diseases and their associated co-morbidities.
- IL-18 and IL-1β are the main targets for treating macrophage activation syndrome, a dangerous condition that can occur in several rheumatic diseases.
- The role of the six newer members of the IL-1 family (IL-36α, IL-36β, IL-36γ, IL-36 receptor antagonist, IL-37 and IL-38) in rheumatic diseases is still being investigated.

The fourth member of the IL-1 family to be discovered was initially named 'IFNγ-inducing factor'[15], but being structurally related to IL-1β, this cytokine's name was later changed to IL-18. IL-33, the fifth member of the IL-1 family, promotes T helper 2 (T_H2) cell responses via its receptor, IL-1 receptor 4 (IL-1R4)[16]. The six other members of the IL-1 family (IL-36α, IL-36β, IL-36γ, IL-36 receptor antagonist (IL-36 Ra), IL-37 and IL-38) were identified by in-silico research methods in the early 2000s[17,18].

This Review provides an update on the prominent biological properties of each member of the IL-1 family, with an emphasis on their roles in rheumatic diseases, including what is known about the processing of the IL-1β precursor by the nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing (NLRP3) inflammasome and the release of IL-1β as an active cytokine in inflammatory diseases. Although less is known about IL-33, IL-37 and the IL-36 subfamily in humans, animal data and data on expression in patients with rheumatic diseases are discussed. The Review concludes with a discussion of the current treatment options for reducing the activities of the IL-1 family using approved drugs, drugs currently in clinical trials and the off-label treatment of rheumatic diseases with IL-1-blocking therapies.

Biology of the IL-1 family

IL-1 family cytokines

The primary functional properties of IL-1 family members are either pro-inflammatory or anti-inflammatory (FIG. 1). IL-1Ra and IL-36Ra are specific for their respective receptors and elicit specific anti-inflammatory effects, whereas IL-37 and IL-38 have non-specific, broad anti-inflammatory effects on both innate and acquired immune responses[19–21]. IL-1 family members are categorized into three subfamilies on the basis of shared receptor or co-receptor binding: IL-1α, IL-1β and IL-33 comprise the IL-1 subfamily and bind the co-receptor IL-1R3; IL-18 and IL-37 form the IL-18 subfamily and bind IL-1R5 (also known as IL-18Rα); and the IL-36 subfamily comprises IL-36α, IL-36β, IL-36γ, IL-36Ra and IL-38, which bind IL-1R6 (also known as IL-36R; FIG. 1). Members of each subfamily have propeptides of similar length, which are cleaved to generate an optimal mature cytokine for receptor binding, the sole exception being IL-1Ra, which has a signal peptide and is readily secreted[22,23].

Each member of the IL-1 family contains a conserved three amino acid consensus sequence, AXD, in which A is an aliphatic amino acid, X is any amino acid and D is aspartate[24]. Nine amino acids forward from the consensus sequence is an N-terminus cleavage site; cleavage at this site enables the optimal folding of the cytokine for receptor binding and activity[24]. For example, nine amino acids forward from the AXD of pro-IL-1β is an alanine at position 117 that forms the site for caspase-1 cleavage[25,26]. Various proteases can generate the N terminus for other IL-1 family members. For example, different neutrophil-derived serine proteases generate three different N termini for pro-IL-33 (REF.[27]) and the enzyme cathepsin S cleaves pro-IL-36γ nine amino acids forward from the AXD site at serine 18 (REF.[28]). How the cell disposes of the accumulated propeptides is unclear, but digestion in autophagosomes is likely[29].

IL-1 family receptors

The IL-1 family of receptors comprises receptor chains that specifically bind each cytokine and co-receptor chains (FIG. 1). IL-1R3 is the co-receptor for IL-1α, IL-1β, IL-33, IL-36α, IL-36β and IL-36γ, all of which have pro-inflammatory functions. By contrast, IL-1R8 (also known as single Ig IL-1-related receptor (SIGIRR)) and IL-1R9 (also known as three immunoglobulin domain-containing IL-1 receptor-related 2 (TIGIRR2)) are co-receptors for IL-37 and IL-38, respectively, which have anti-inflammatory functions. Interestingly, *IL1RAPL2*, which encodes IL-1R9, is located on the X chromosome, and mutations in *IL1RAPL2* result in severe X-linked intellectual disability[30]. Although IL-1R9 might be required for cognitive function[31], a role for IL-1R9 in suppressing inflammation is also likely. IL-1R10 (also known as TIGIRR1) is an orphan receptor with a similar structure to IL-1R9, which suggests that this receptor might be a co-receptor for a member of the IL-1 family, perhaps IL-33 (REFS[32,33]).

A fundamental process in IL-1 family signalling is the formation of a heterotrimeric complex containing the ligand, receptor and co-receptor[34]. For example, IL-1β binds to its specific receptor (IL-1R1) and co-receptor (IL-1R3), and this trimeric complex triggers a pro-inflammatory signal (FIG. 2a). Similarly, IL-18 binds to its receptor (IL-1R5) and co-receptor (IL-1R7) to deliver a pro-inflammatory signal[35,36]. By contrast, IL-37 binds to its receptor (IL-1R5) but forms a complex with the co-receptor IL-1R8, thereby eliciting an anti-inflammatory signal[19,37,38]. However, the anti-inflammatory properties of IL-37 were not evident when recombinant human IL-37 was administered to IL-1R8-deficient mice challenged with a variety of inflammatory conditions[39–41], suggesting that the co-receptor imparts the biological function of the cytokine.

With the exception of red blood cells, most cells express IL-1R1 and the co-receptor IL-1R3 (REFS[42,43]). Upon binding to IL-1β (or IL-1α), a conformational change occurs in IL-1R1, which allows IL-1R3 to bind[44], forming a heterotrimeric complex (FIG. 2a). IL-1R3 can also make contact with the cytokine itself within the complex[34,44,45]; the aspartate at position 145 in both IL-1β and IL-1α is critical for binding to IL-1R3, forming a

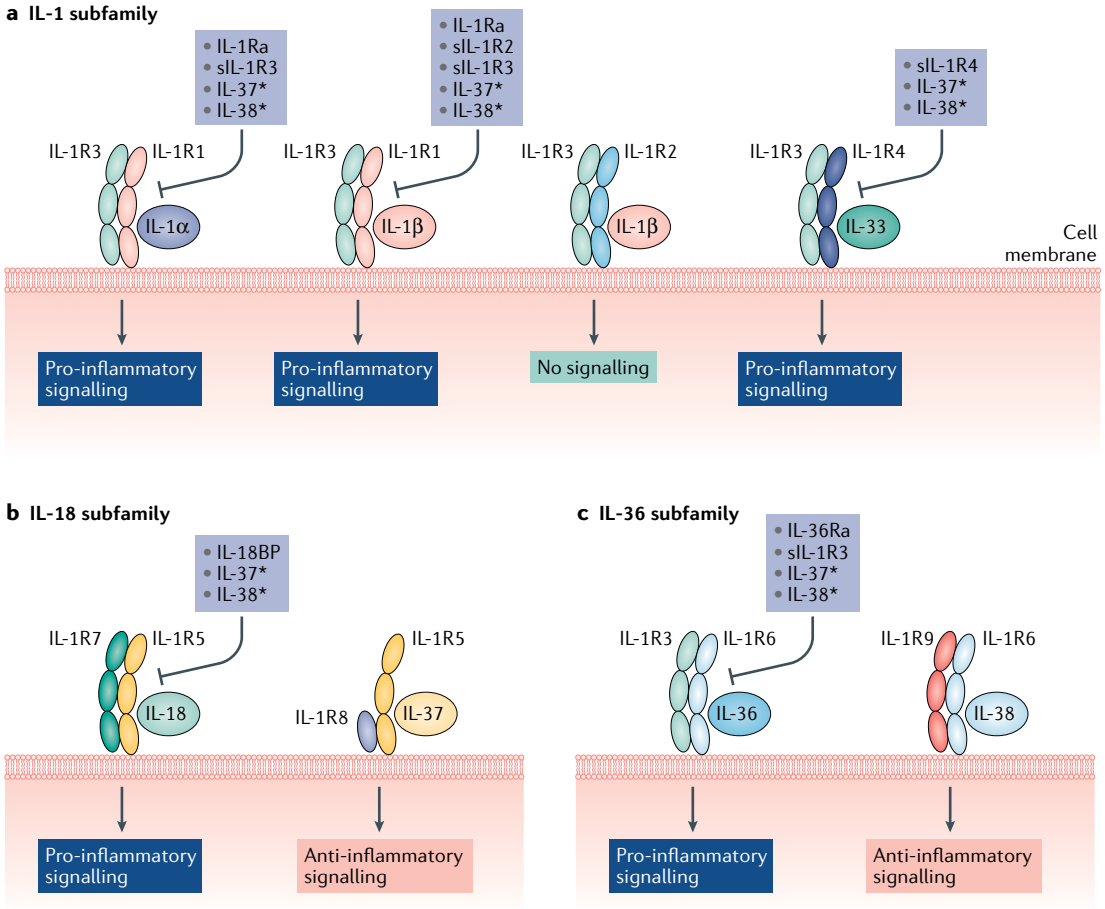


Fig. 1 | **IL-1 cytokine subfamilies and receptors.** The IL-1 family of cytokines can be divided into three subfamilies on the basis of shared receptor and co-receptor binding: the IL-1 subfamily, the IL-18 subfamily and the IL-36 subfamily. **a** | The IL-1 subfamily consists of IL-1α, IL-1β and IL-33, which share the co-receptor IL-1 receptor 3 (IL-1R3). Cytokines of the IL-1 subfamily promote pro-inflammatory signalling pathways that induce the production of other cytokines and chemokines. By contrast, IL-1 receptor antagonist (IL-1Ra) specifically reduces the activities of IL-1α and IL-1β. The activity of IL-1β can also be reduced by binding to the decoy receptor IL-1R2, which produces no downstream signal, instead of to IL-1R1. Soluble versions of IL-1 family receptors also exist, such as soluble IL-1R2 (sIL-1R2) and sIL-R3. sIL-1R2 specifically binds and neutralizes IL-1β; however, the affinity of sIL-1R2 for IL-1β increases several-fold in the presence of sIL-1R3. **b** | The IL-18 subfamily consists of IL-18 and IL-37, which share the receptor IL-1R5. IL-18 induces pro-inflammatory signalling pathways. IL-18 is specifically antagonized by IL-18 binding protein (IL-18BP), which has an unusually high affinity for IL-18. Unlike IL-18, which binds to the co-receptor IL-1R7, IL-37 promotes anti-inflammatory effects via the co-receptor IL-1R8. **c** | The IL-36 subfamily consists of IL-36α, IL-36β, IL-36γ and IL-38, which share IL-1R6. IL-36 cytokines (shown in the figure as IL-36) promote pro-inflammatory signalling pathways that are specifically antagonized by IL-36 receptor antagonist (IL-36Ra). Similar to IL-37, IL-38 is anti-inflammatory. *IL-37 and IL-38 have broad ranging anti-inflammatory properties that include reducing the production of other IL-1 family members or indirectly inhibiting their activities.

hydrogen bond with the serine at position 205 in IL-1R3 (REFS[34,45]). The formation of the heterotrimeric complex brings the intracellular domains of IL-1R1 and IL-1R3 into close proximity, which enables the Toll/IL-1 receptor (TIR) domains (BOX 1) to recruit MYD88 and triggers a subsequent cascade of kinases that results in the cell's pro-inflammatory state. By contrast, when IL-1Ra binds to IL-1R1, a different conformational change occurs. Upon binding of IL-1Ra to IL-1R1, a complex with IL-1R3 fails to form, no TIR domains approximate and there is no signal[45] (FIG. 2b). IL-1Ra binds to IL-1R1 with a higher affinity than either IL-1α or IL-1β, making IL-1Ra a highly effective receptor antagonist, although only a single mutation in IL-1Ra is required to convert this antagonist into an agonist[46].

Similarly, IL-1R2, a decoy receptor that has no cytoplasmic domain[47], functions to prevent IL-1 cytokine activity, particularly that of IL-1β. As an integral membrane receptor, IL-1R2 binds IL-1β, undergoes a conformational change and forms a complex with IL-1R3; however, as IL-1R2 lacks a TIR domain, there is no signal (FIG. 2c). The extracellular domain of IL-1R2 can be released from the cell by proteolytic cleavage; this soluble form of IL-1R2 binds and neutralizes IL-1β in the extracellular milieu. Moreover, the neutralization of IL-1β by soluble IL-1R2 is greatly enhanced when it forms a complex with soluble IL-1R3 (REF.[48]). Soluble IL-1R3 can also form complexes with soluble IL-1R1, as well as with soluble IL-1R4 and with IL-1R6, neutralizing IL-1α, IL-33, IL-36α, IL-36β and IL-36γ[49]. Soluble IL-1R4,

which specifically binds IL-33 (REF.[50]), is also present in the human circulation and potentially affects disease outcome in graft-versus-host disease[51]. Although IL-18 binding protein (IL-18BP), being a secreted protein, is not a soluble receptor, it functions as a soluble receptor and has a high affinity for IL-18; IL-18BP is discussed in further detail below.

IL-1 subfamily

IL-1α

Evolutionarily, IL-1α is the oldest member of the IL-1 family and is thought to have functioned as a transcription factor[52,53] in early organisms before cell surface receptors evolved. IL-1α is structurally related to fibroblast growth factor and is involved in similar repair processes in the skin[54]. Five characteristics of IL-1α distinguish this cytokine from IL-1β: pro-IL-1α is constitutively present in mesenchymal cells throughout the body in healthy individuals[55,56], whereas pro-IL-1β is only constitutive in resident macrophages[57]; pro-IL-1α is active[58,59], whereas pro-IL-1β requires processing via caspase-1 to become active[25]; IL-1α is functional as an integral membrane protein[60,61], whereas IL-1β is not present at the cell membrane[62]; IL-1α is active in the nucleus[52,54,63], whereas IL-1β is not found in the nucleus; and IL-1α is rarely reported in the circulation in disease states, whereas IL-1β is found in the circulation in both health[64,65] and disease[66,67]. Therefore, the role of IL-1α in disease is primarily local, not systemic. In fact, within the cell, pro-IL-1α binds to IL-1R2, which might prevent the release of this cytokine[68].

IL-1α in osteoarthritis. Worldwide, osteoarthritis (OA) is the most common reason for individuals to seek the advice of a physician for painful joints, particularly in the ageing population[69]. Several studies have shown that IL-1α, as well as IL-1β and other members of the IL-1 family, are present in the synovial fluid and the synovial membranes of patients with OA[70,71]. However, results from mouse models of OA differ as to whether there is a causative role of IL-1α or IL-1β[72]. Pro-IL-1α is constitutively present in chondrocytes, which are embedded in the cartilage, making it difficult for antibodies to access them. In cultured articular cartilage from pigs, mechanical stress (a contributing factor to OA) induces the production of IL-1α and the release of proteases[73]. For example, the binding of IL-1α to IL-1R1 initiates the activation of proteases and the degradation of cartilage[73]. Of the many proteases released by the application of mechanical stress to cartilage, a disintegrin and metalloproteinase with thrombospondin 5 (ADAMTS5) is prominent and degrades the matrix glycoprotein aggrecan[74]. In animal models of rheumatoid arthritis (RA), blocking various members of the IL-1 family reduces the loss of proteoglycans in cartilage[75–77], and patients with RA treated with daily anakinra have reduced joint space narrowing[78]. To date, there have been no studies in patients with OA in which IL-1α is specifically targeted.

IL-1α in Kawasaki disease. Kawasaki disease is a form of vasculitis in which IL-1α is likely to be involved, as it is constitutively present in the endothelium. During any endothelial cell stress, such as in systemic lupus

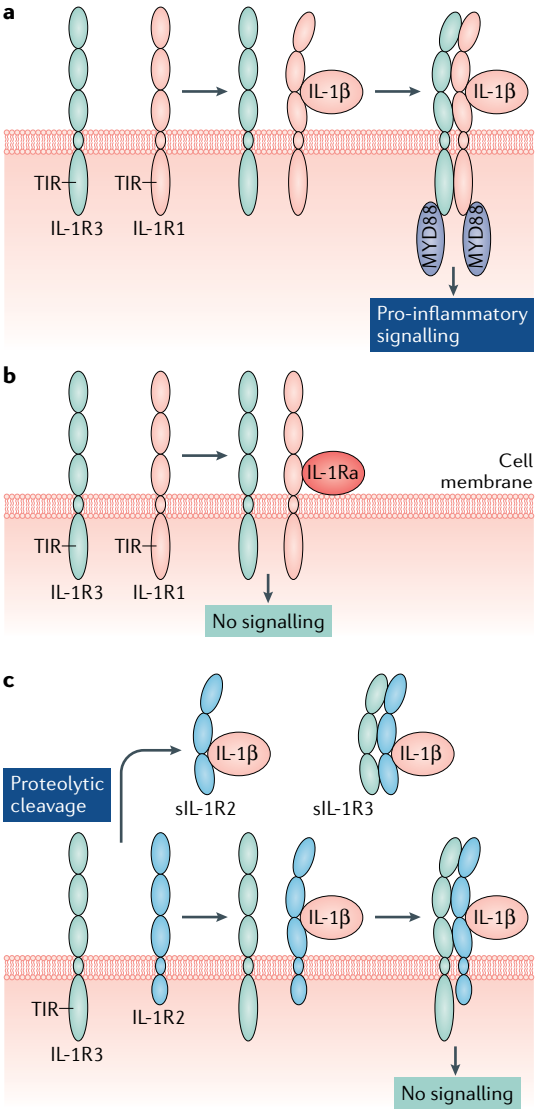


Fig. 2 | **IL-1 receptor signalling. a** | IL-1 receptor 1 (IL-1R1) and the co-receptor IL-1R3 both exist as integral membrane proteins. Upon binding of IL-1β to IL-1R1, a conformational change in the receptor occurs such that IL-1β binds to the third immunoglobulin domain of the receptor. This conformational change in IL-1R1 enables the binding of IL-1R3 to create a heterotrimeric complex. The close proximity of the extracellular domains of IL-1R1 and IL-1R3 results in the bringing together of the intracellular Toll/IL-1 receptor (TIR) domains, which leads to the recruitment of MYD88 and the initiation of a pro-inflammatory signalling pathway. **b** | When IL-1 receptor antagonist (IL-1Ra) binds to IL-1R1, it undergoes a different conformational change to that which occurs upon binding IL-1β. This conformational change does not enable the recruitment of IL-1R3, and no signalling occurs. **c** | IL-1R2 is an integral membrane protein that lacks an intracellular TIR domain, and therefore functions as a decoy receptor. IL-1β binds to IL-1R2 in a similar manner to IL-1R1 and recruits IL-1R3, but MYD88 cannot bind to the intracellular domain of IL-1R2 and no signalling can take place. A soluble form of IL-1R2 (sIL-1R2) can be formed by proteolytic cleavage of IL-1R2, and can bind IL-1β and neutralize this cytokine. sIL-1R2 can also form a complex with sIL-1R3, which increases its ability to neutralize IL-1β.

Box 1 | **Similarities between IL-1R and TLR signalling**

The 50 amino acid Toll/IL-1 receptor (TIR) domain that is present in each member of the IL-1 receptor (IL-1R) family is nearly identical to the TIR domains in each member of the Toll-like receptor (TLR) family. The discovery that related the two families was made in 1991 by comparison of IL-1R1 with the Toll protein of *Drosophila*[358], a finding that was reported many years before the identification of TLRs. Within 1 year of the discovery, the TIR domain of IL-1R1 was shown to be essential for IL-1 activities[359]. Thus, fundamental inflammatory responses such as the induction of cyclooxygenase 2 and production of multiple cytokines and chemokines can be promoted by IL-1, as well as by TLR ligands. Although TLRs trigger inflammation in response to bacteria, microbial products, viruses, nucleic acids and damage-associated molecular patterns, blockade of TLR4 for the treatment of septic shock failed to be effective in a large trial[360]. By contrast, blockade of IL-1R1 with anakinra is effective for the treatment of a broad spectrum of both rheumatic diseases and other diseases (Supplementary Table S1). The failure of antibodies that block TLRs in clinical trials might relate to preclinical data in the mouse.

erythematosus (SLE) or in Kawasaki disease, large apoptotic bodies containing active IL-1α are released[79]. Deficiency in IL-1α was also protective in a mouse model of Kawasaki disease[80]. Several case studies have revealed a substantial benefit of anakinra treatment, which blocks both IL-1α and IL-1β in patients with Kawasaki disease, particularly in those who are resistant to intravenous IgG therapy[81,82]. A case series that included 11 children with Kawasaki disease described a rapid and marked clinical improvement, a reduction in coronary artery dilation and a fall in plasma C-reactive protein (CRP) concentration following treatment with anakinra[82]. In another case study, the authors reported a reduction in coronary aneurysms following anakinra treatment[83]. An open label trial that uses increasing doses of anakinra to treat Kawasaki disease is currently being conducted[84], although no studies have investigated specifically blocking IL-1α alone in Kawasaki disease.

IL-1α in other rheumatic diseases. Unlike IL-1β and IL-18, concentrations of circulating IL-1α can be below the detection limit and therefore correlations between concentrations of circulating IL-1α and disease can be variable. However, the availability of a neutralizing human anti-IL-1α antibody (currently used to treat cancer and inflammatory skin conditions[85–90]) has enabled IL-1α to be studied in rheumatic diseases via selective blockade. IL-1α is present in keratinocytes, and anti-IL-1α has shown benefit in hidradenitis suppurativa[90,91] and pustular psoriasis[86]; however, whether blocking IL-1α in patients with psoriatic arthritis (PsA) would reduce joint disease remains unknown. IL-1α is also associated with fibrotic processes in systemic sclerosis (SSc)[92,93], but to date there have to our knowledge been no clinical trials of anakinra or anti-IL-1α in SSc.

A role for IL-1α in the pathogenesis of RA or gout has yet to be discovered; animal studies uncovered no important role for IL-1α in commonly used models of RA (such as collagen-induced arthritis (CIA)) or gout[94,95]. However, naturally occurring anti-IL-1α autoantibodies are present at higher titres in patients with polyarthritis than in patients with RA and are associated with less severe forms of arthritis[96,97], suggesting that these autoantibodies might have a protective function. By contrast, IL-1α might have a role in myositis; encouraging results from IL-1 blockade studies in a mouse model of polymyositis[98,99] led to a mechanistic study of anakinra in 15 patients with refractory myositis, 7 of whom responded to treatment[100,101]. Similarly, increased concentrations of IL-1α are present in the circulation of patients with SLE[102], and although to our knowledge no formal trial has been conducted to specifically block IL-1α in patients with SLE, a preliminary trial of anakinra in four patients with lupus arthritis reported some benefits[103]. Patients with SLE who presented with macrophage activation syndrome (MAS) also showed improvement following anakinra treatment[104,105]. A human anti-IL-1α monoclonal antibody that is currently being studied in cancer[87] and dermatological diseases[90] is likely to be studied in rheumatic diseases such as SLE and PsA in the future.

IL-1β

IL-1β is the most frequently studied member of the IL-1 family, with properties that are relevant to several rheumatic diseases[106]. The relevance of IL-1β for rheumatic diseases is attributable to the pathological infiltration of myeloid cells into joints. IL-1β is a product of myeloid-derived cells; expression of *IL1B* is low or absent in freshly obtained human blood monocytes but increases upon stimulation with Toll-like receptor (TLR) ligands or with IL-1α or IL-1β[107]. A 2019 study[108], in which synovial tissues from patients with arthritis, characterized as OA, leukocyte-rich RA or leukocyte-poor RA, were examined for fibroblasts, T cells, B cells and monocytes using single-cell RNA sequencing, mass cytometry, bulk RNA sequencing and flow cytometry, found that in monocytes, but not in T cells, IL-1β dominated, with high Z scores in all three types of arthritis[108]. Monocytes were enriched for pathways involved in TLR signalling, myeloid leukocyte activation, MYD88-dependent signalling, cytokine production and autophagosome assembly, each of which is known for involvement in IL-1β activation. The results of this study[108] support the efficacy of IL-1β blockade in RA, as well as a role for IL-1β in OA. The importance of IL-1β in rheumatic diseases (as well as in comorbidities such as type 2 diabetes mellitus, atherosclerosis and heart failure) was highlighted in the results of the Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS), a worldwide randomized, placebo-controlled study in 10,061 individuals[109]. Canakinumab, a neutralizing human monoclonal antibody targeting IL-1β, was used to test the hypothesis that blocking IL-1β would reduce the possibility of a second cardiovascular event in patients who had survived a first heart attack or stroke. The 4-year study met its primary and secondary endpoints and validated decades of in vitro, animal, preclinical and clinical studies on IL-1β, particularly on its role in the pathogenesis of atherosclerosis[110]. The CANTOS trial[109] also confirmed a role for IL-1β in the pathogenesis of gout[111], OA[112], type 2 diabetes mellitus[113–115] and heart failure[116,117]. In addition, post-hoc analysis showed a highly statistically significant reduction in the incidence of and survival from lung cancer[118], although this result was not entirely unexpected[119,120].

Synthesis, processing and release of IL-1β. The processing of IL-1β from its inactive precursor and the secretion of the active cytokine is complex, and several mechanisms have been reported. The initial activation of monocytes or macrophages is often termed 'signal 1' and can be caused by TLR agonists, IL-1 itself (IL-1α or IL-1β), immune complexes, adjuvants or danger-associated molecular patterns. Regardless of the initiating stimulus, once the cell has been activated, pro-IL-1β accumulates in the cytosol (FIG. 3a) and is then secreted by one of five different methods. Activation of the NLRP3 inflammasome is often termed signal 2, and initiates the cleavage of pro-IL-1β by caspase-1. Activation of the P2X7 receptor by ATP causes the potassium channel to open. As intracellular potassium concentrations fall, the NLRP3 inflammasome is activated and pro-caspase-1 is converted into active caspase-1. Mature IL-1β can then be released from specialized secretory lysosomes as a result of a calcium influx[121–124]. Another mechanism by which IL-1β can exit the cell is via exosomes[125]. They contain NLRP3, pro-IL-1β, caspase-1 and major histocompatibility complex (MHC) class II molecules, and rely on caspase-1 for the processing of IL-1β. In many ways, these exosomes are similar to secretory lysosomes, including the requirement for calcium influx. The unique aspect of these exosomes is the release of MHC class II molecules into the extracellular space along with IL-1β.

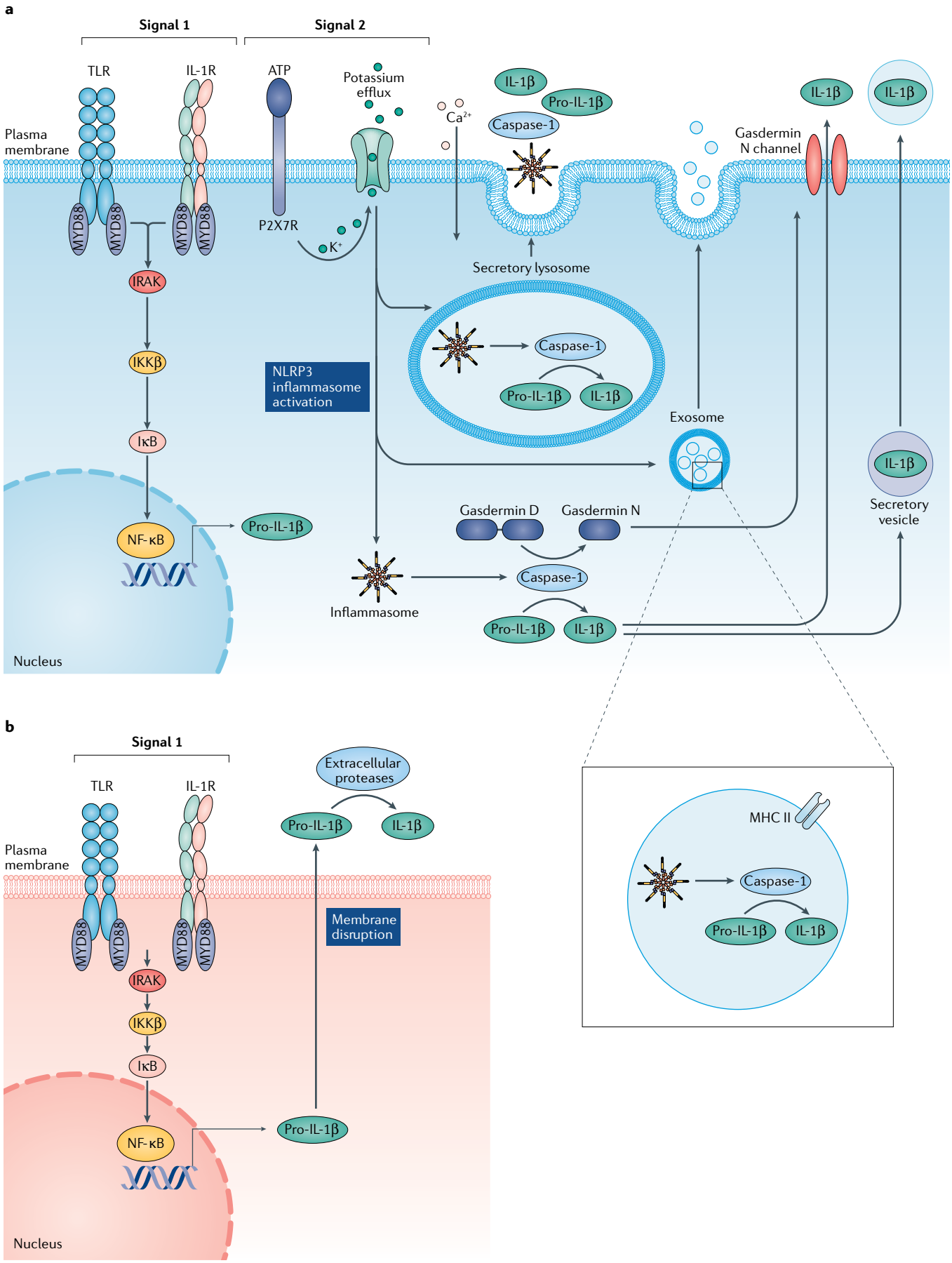
In the past few years, interest has focused on the role of the NLRP3-dependent gasdermin N channel in the secretion of IL-1β[126–128] and on caspase-1-dependent inflammation as a result of macrophage cell death by pyroptosis. A 2018 study has also described release of IL-1β via the gasdermin N channel that is dependent on caspase-8 (REF.[129]). Although several studies in mice, mouse cells and cell lines have suggested that caspase-1-dependent release of IL-1β via the gasdermin N channel results in cell death by pyroptosis[130–132], the release of IL-1β can also take place in human blood monocytes without cell death occurring[124,127,133]. The gasdermin N channel forms following caspase-1 cleavage of the gasdermin D precursor to produce gasdermin N, which polymerizes to form a channel through which mature IL-1β exits the cell. In this model, caspase-1 serves two functions: processing of pro-IL-1β into IL-1β and processing of the gasdermin D precursor to gasdermin N. Depending on the activation state of the monocyte, mature IL-1β can be shed from the cell in plasma membrane microvesicles[124] (as occurs in the absence of reactive oxygen species (ROS)), or IL-1β can exit the cell via the gasdermin D channel[124] (as occurs in the presence of ROS).

Under conditions of stress, such as hypoxia, the plasma membrane can lose integrity and pro-IL-1β can be released into the extracellular compartment along with lactate dehydrogenase (LDH), which is an indicator of loss of membrane integrity, and other intracellular components (FIG. 3b). Once outside the cell, extracellular proteases cleave pro-IL-1β near to the caspase-1 cleavage site, generating mature IL-1β[134,135]. This mechanism is independent of caspase-1 and was particularly relevant in the joints of mice with gouty arthritis, where neutrophil proteases predominate[94], and might be relevant in human gout. Neutrophil elastase and protease 3 cleave pro-IL-1β at sites within a few amino acids of the caspase-1 cleavage site, and α1-antitrypsin, which inhibits neutrophil elastase and protease 3, markedly reduced the extracellular processing of pro-IL-1β in these mice[94].

IL-1β in hereditary autoinflammatory diseases. Autoinflammatory diseases are monogenic syndromes, but, unlike autoimmune diseases such as RA, there is only a small, if any, role for dysfunctional autoreactive T cells or autoantibodies in the pathogenesis of autoinflammatory diseases[136]. Instead, dysfunctional macrophages account for the inflammation that occurs in these diseases[137] owing to a loss of control in the processing and release of active IL-1β (REFS[124,138,139]). Autoinflammatory diseases caused by mutations in *NLRP3* are collectively termed cryopyrin-associated periodic syndromes (CAPS). The NLRP3 inflammasome is the dominant route for the processing and secretion of IL-1β (FIG. 3a), and mutations in *NLRP3* in patients with CAPS are gain-of-function, meaning that these individuals have chronic, systemic and local inflammation due to active IL-1β[140]. The symptoms of CAPS are highly responsive to specific IL-1β neutralization[66,67] or IL-1R1 blockade with anakinra (Supplementary Table S1). Familial Mediterranean fever (FMF) is another IL-1β-mediated, monogenic autoinflammatory disease, but in FMF the mutation is present in *MEFV*, which encodes pyrin[141,142]. Mutations in pyrin result in a loss of control of NLRP3, a mechanism distinct from the mechanism in CAPS. Although these are rare disorders, the clinical spectrum of disease and haematological and metabolic abnormalities that occur are common to most acute and chronic inflammatory conditions. However, concentrations of circulating IL-1β are low in autoinflammatory diseases and do not make a reliable biomarker. Instead, the release of IL-1β (but not TNF) from monocytes from patients with autoinflammatory diseases is consistently high in vitro[143–146].

IL-1β in gout. Gout is a uniquely IL-1β-mediated disease. An acute flare of gout begins with the engulfment of monosodium urate (MSU) crystals by synovial macrophages, which produce IL-1β. The MSU crystals themselves are only weak inducers of IL-1β[147] and two signals are required to produce a strong response. Although TLR4 can provide such a signal[147], a role for TLR4 in the pathogenesis of gout is clinically unlikely; rather, fatty acids signalling via TLR2 can provide the signal in gout for the synthesis of pro-IL-1β (REF.[95]), which might account for the association between gout flares and dietary factors. Once the fatty acids via TLR2 signal IL-1β gene expression and pro-IL-1β synthesis, MSU crystals are engulfed by synovial macrophages, NLRP3 is activated and caspase-1 cleaves pro-IL-1β to release mature IL-1β into the synovial space; pain is caused by IL-1β-mediated induction of PGE_2 via increased cyclooxygenase 2 (COX2)[148].

Gout is responsive to anakinra[149,150], rilonacept (a soluble IL-1 receptor that neutralizes IL-1α and


a
Signal 1
Signal 2
TLR
IL-1R
ATP
Potassium efflux
Ca2+
IL-1β
Pro-IL-1β
Caspase-1
Gasdermin N channel
Plasma membrane
MYD88
P2X7R
K+
Secretory lysosome
IRAK
IKKβ
IκB
NLRP3 inflammasome activation
Exosome
Secretory vesicle
NF-κB
Pro-IL-1β
Gasdermin D
Gasdermin N
Inflammasome
Nucleus
b
Signal 1
Extracellular proteases
Membrane disruption
MHC II

◀ Fig. 3 | **Expression, synthesis, processing and release of IL-1β. a** | Cell surface IL-1 receptor (IL-1R) or Toll-like receptors (TLRs) are activated by their respective ligands (known as signal 1). The Toll/IL-1 receptor (TIR) domains of these receptors recruit MYD88, which initiates a cascade of four phosphorylated kinases called IL-1R-associated kinases (IRAKs), followed by the phosphorylation of IκB kinase β (IKKβ), IκB and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), resulting in transcription of *IL1B*. *IL1B* mRNA is translated into pro-IL-1β, which accumulates in the cytosol. A second signal is required for pro-IL-1β to be transformed into active cytokine. Extracellular ATP binds to P2X7 receptor (P2X7R), which causes potassium to exit the cell through the potassium channel. As intracellular potassium concentrations fall, the nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing (NLRP3) inflammasome is activated and pro-caspase-1 is cleaved into active caspase-1. Pro-IL-1β can be cleaved by caspase-1 in the cytosol or in endosomal compartments, and can be released through several different routes. First, as intracellular calcium increases in the cell, secretory lysosomes can release mature, active IL-1β into the extracellular compartment, together with components of the inflammasome. Second, caspase-1 can cleave the N terminus of gasdermin D to yield subunits of gasdermin N, which form a pyroptotic pore. Mature IL-1β can then exit the cell through this pyroptotic pore. Third, mature IL-1β can be shed from the cell in plasma membrane microvesicles. Fourth, microvesicular body-derived exosomes containing NLRP3, pro-IL-1β, caspase-1 and major histocompatibility complex (MHC) class II molecules can be exported from the cell in response to calcium influx. **b** | Under cellular stress conditions such as hypoxia, necrosis or misfolding of intracellular proteins, the plasma membrane can lose integrity, causing lactate dehydrogenase, pro-IL-1β and other intracellular content to be released into the extracellular compartment. Extracellular proteases in the inflammatory milieu then cleave pro-IL-1β near the caspase-1 site to generate mature IL-1β.

IL-1β (REF.[151]) and canakinumab[111]. The results of a non-inferiority trial of anakinra or standard-of-care therapy (prednisone, naproxen or colchicine) for acute flares of gout was published in 2019 (REF.[152]). In this study, similar reductions in pain scores were found for patients receiving anakinra and those receiving standard-of-care therapy[152]. However, standard-of-care therapy for gout has known drawbacks for patients with gout who also have type 2 diabetes mellitus (as corticosteroids increase blood glucose) or poor renal function (as NSAIDs worsen kidney function), whereas anakinra is safe for patients with these comorbidities. In the CANTOS trial population[109], patients who received canakinumab had fewer gout flares than those who received placebo. An alternative approach to targeting IL-1β is via direct inhibition of the NLRP3 inflammasome. This approach has been validated in patients with acute gout flares in early-phase clinical trials of dapansutrile, an orally active specific inhibitor of NLRP3 (REF.[153]). Patients receiving dapansutrile had dose-dependently reduced pain and reduced concentrations of circulating IL-1β, IL-6 and CRP after 3 days of treatment[154,155]. The future of targeting IL-1β could rest with the continued development and assessment of such oral NLRP3 inflammasome inhibitors.

The fact that gout is a systemic disease is often overlooked; gouty arthritis is only the tip of the iceberg when it comes to systemic complications of hyperuricaemia. Individuals with hyperuricaemia have chronic inflammation, and many studies have linked hyperuricaemia to increased morbidity and mortality owing to hypertension, atherosclerosis, chronic kidney disease and type 2 diabetes mellitus[156–158]. Mechanistically, high concentrations of serum uric acid suppress the production of endogenous IL-1Ra[159,160]. As a result of low concentrations of circulating IL-1Ra, concentrations of IL-1β increase; monocytes from individuals with hyperuricaemia release more IL-1β than monocytes from individuals without hyperuricaemia[159,160]. Compared with cells primed with vehicle only, transcriptomic analysis of urate-primed monocytes revealed increased mechanistic target of rapamycin (mTOR) signalling and decreased autophagic activity[160]. Although persons with hyperuricaemia are at risk of recurrent gout flares, they are also at risk of cardiovascular diseases. The suppression of endogenous IL-1Ra probably contributes to both risks.

IL-1β in systemic-onset juvenile idiopathic arthritis and adult-onset Still's disease. Systemic-onset juvenile idiopathic arthritis (sJIA) and adult-onset Still's disease (AoSD) are often considered to be the same disease, and sJIA is sometimes called juvenile Still's disease. In both AoSD and sJIA, IL-1β is either elevated in the circulation or released from cultured monocytes ex vivo[161,162]. sJIA can be effectively treated by blocking IL-1R1 with anakinra[161,163] or by neutralizing IL-1β with canakinumab[164,165]. Canakinumab is currently approved in the USA and in Europe for the treatment of sJIA (see Supplementary Table S2). An oral histone deacetylase inhibitor, givinostat, has also been trialled for sJIA[166]; in preclinical and early-phase clinical studies, givinostat reduced the secretion of IL-1β[167,168]. Givinostat is in European trials for X-linked muscular dystrophy and graft-versus-host disease.

The use of anakinra to treat refractory AoSD began 16 years ago[162,169,170], and canakinumab and rilonacept are also effective for treating AoSD[171,172]. Canakinumab is marketed in the UK and Hungary for AoSD (see Supplementary Table S2) and today, IL-1 inhibitors are the standard-of-care therapy for this disease[171–173]. Blocking TNF is not effective for the treatment of AoSD, but anti-IL-6 receptor antibodies have been used in patients who do not achieve good disease control with anakinra[173]. Many of the systemic manifestations of AoSD (FIG. 4), such as fever, neutrophilia and increased IL-6 and CRP, are rapidly reduced and the reduction sustained with daily doses of anakinra or monthly doses of canakinumab (Supplementary Table S1). Hyperferritinaemia is often present and is more specific to AoSD than increased CRP. A salmon-coloured macular rash is also uniquely observed in AoSD. These two unique characteristics of AoSD rapidly resolve upon IL-1β blockade; moreover, patients with AoSD can develop painful pericarditis and myocarditis, which also respond to IL-1β blockade with anakinra[173,174]. Pulmonary hypertension has also been observed in patients with AoSD[162], but it is unclear whether pulmonary hypertension responds to IL-1β blockade. Uveitis in these patients can be treated with anakinra; severe uveitis has been successfully treated with the anti-IL-1β monoclonal antibody givokizumab[175]. MAS is also an important consideration for patients with sJIA or AoSD. Although blockade of IL-1R1 with anakinra is effective for treating MAS, the neutralization of IL-18 with IL-18BP might be the best treatment option for treating MAS, and has been used in a pilot trial in patients with AoSD[176,177]. Blocking IL-18 with IL-18BP in AoSD is consistent with IL-18's role as a pro-inflammatory cytokine induced by IL-1β (FIG. 5).

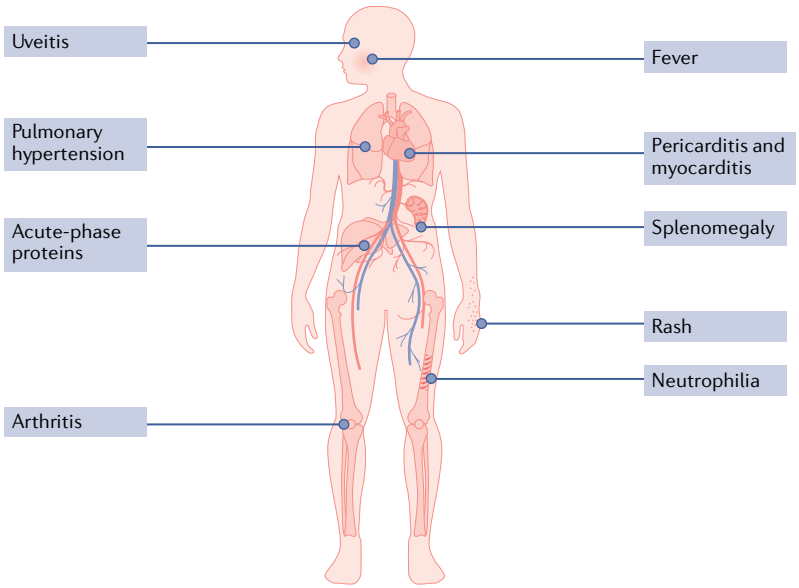


Fig. 4 | **Systemic manifestations of adult-onset Still's disease.** Systemic manifestations of adult-onset Still's disease (AoSD) include uveitis, fever, pulmonary hypertension, pericarditis, splenomegaly, arthritis, rash, neutrophilia and an increase in acute phase proteins. The most characteristic sign of AoSD is a salmon pink rash, although this rash can also occur in systemic-onset juvenile idiopathic arthritis (sJIA). Recurrent, daily fevers are also typical in AoSD, particularly during the early phases of the disease. Pericarditis is commonly observed, whereas myocarditis is less often present. Uveitis can also occur. Splenomegaly occurs in most individuals with AoSD owing to extramedullary haematopoietic expansion. Of the systemic manifestations of AoSD, neutrophilia is the most consistent. Compared with the systemic manifestations of sJIA, arthritis is the most variable manifestation of AoSD, and can range from affecting a few joints to affecting many joints severely. Idiopathic pulmonary hypertension is a rare but dangerous complication in AoSD.

As sJIA and AoSD are effectively controlled by IL-1-blocking therapies, what then are the pathogenic mechanisms that promote the production of IL-1β? At present, no specific mutations in *NLRP3* have been discovered that would account for the increased production of IL-1β in these diseases. Several single-nucleotide polymorphisms (SNPs) in genes encoding potassium channels[161] are associated with AoSD, and a comprehensive study identified *HLA-DRB1*11* and variants of the MHC class II locus as risk factors for sJIA[178]. A SNP in the promoter of *MIF*, which encodes macrophage migration inhibitory factor, has also been linked to AoSD[179], as have rare coding variants in IL-1-related genes[180]. Monocytes from patients with sJIA release increased amounts of IL-1β[161], and mechanistically, aryl hydrocarbon receptor is thought to be involved in the release of IL-1β in sJIA[181]. Overall, AoSD and sJIA seem to be IL-1β-mediated diseases, but the exact mechanisms involved require further study to be fully elucidated.

IL-1β in osteoarthritis. Despite mixed results in data from mice and humans, more than any other cytokine, IL-1β has been linked to cartilage loss and to the pathogenesis of destructive OA. For example, in chondrocytes from patients with OA undergoing knee replacement surgery, the production of nitric oxide (a sign of activation) was reduced by the addition of an NLRP3 inhibitor to the culture conditions to suppress the release of IL-1β[182]. Early in the disease process, myeloid cells infiltrating the synovial space function as a source of IL-1β[183,184]. As the disease progresses, there are fewer infiltrating myeloid cells and synovial lining cells become the most likely source of IL-1β[185]. In humans, the presence of IL-1β is firmly associated with OA. In a study of cytokine production in 82 individuals aged 90 years, after correcting for sex and BMI, those who produced the lowest amounts of IL-1β had the highest chance of being free of OA[186]. In another study that looked at cytokine production in peripheral blood mononuclear cells (PBMCs) from 436 women in the Framingham cohort, high amounts of IL-1β were associated with the presence of knee osteophytes and joint space narrowing[187]. Several studies have provided evidence to support a primary role for IL-1β in OA pain. IL-1β induces COX2, which causes an increase in PGE_2 (REF.[188]), thereby potentially lowering an individual's pain threshold. The use of oral COX2 inhibitors to relieve OA pain is widespread, and these drugs potentially target pain by reducing IL-1β-induced production of PGE_2. IL-1Ra is also found in the synovial fluid of patients with OA[70,183,189,190]; in a mouse model of OA, synovial IL-1Ra inhibited IL-1β-induced COX2 production in synovial lining cells, as well as IL-1α-mediated cartilage degradation[191].

Lutikizumab (ABT-981), a novel antibody with the dual function of neutralizing both IL-1α and IL-1β, was tested in a randomized, placebo-controlled trial of erosive hand OA[192]. The antibody was administered subcutaneously every 2 weeks but, after 16 weeks, no difference in pain or joint imaging scores was seen between the treatment group and the placebo group[192]. However, in a phase I study of knee OA, lutikizumab reduced the number of circulating neutrophils and the amount of CRP, as well as metalloproteinase-derived collagen breakdown products, which indicated a reduction in destructive joint inflammation[193]. Increasing doses of lutikizumab were subsequently tested in a randomized, placebo-controlled trial of 350 patients with Kellgren–Lawrence grade II–III knee OA[194] in which pain was assessed using the Western Ontario and McMaster University (WOMAC) pain score. At week 16, a statistically significant reduction in pain was recorded in those patients treated with 100 mg lutikizumab compared with those who received placebo; however, after week 16, differences in pain were no longer significantly different between those receiving lutikizumab and those receiving placebo. Overall, it seems that lutikizumab is not effective for the treatment of OA.

Two trials have tested the effects of intra-articular administration of anakinra in patients with knee OA[112,195]. In the first trial[112], a single dose of 150 mg of anakinra was used in 13 patients, and total pain and the WOMAC functional index scores were recorded for 3 months. Improvements in the total pain and WOMAC scores were reported up to month 3 (REF.[112]). In this trial, the half-life of anakinra in the joint was ~4 h, which might explain the lack of long-term benefits of intra-articular anakinra. In the second trial, a randomized, placebo-controlled trial, 170 patients received a single dose of either 50 mg or 150 mg of anakinra intra-articularly. After 4 weeks, there was no difference between placebo

and anakinra in pain reduction[195]. The authors attributed the lack of response to the short duration (4 h) of anakinra in the joint. Delivery of IL-1Ra by various gene therapy vectors has also been trialled in humans with promising responses[196]. A monoclonal antibody targeting IL-1R1 (AMG 108) has also been tested in a randomized controlled study of 160 patients with knee OA[197]. The antibody was administered either subcutaneously or intravenously every 4 weeks and joint pain was assessed after 12 weeks using the WOMAC pain score. Patients with the highest amount of knee pain treated with AMG 108 had a reduction in their median WOMAC pain score of −63 compared with a reduction of −37 in those treated with placebo[197].

Data from the CANTOS trial[109] also supports a role for IL-1β in OA. The CANTOS cohort included older individuals and those with high BMI, each of which is a risk factor for OA. Although not part of the design of the

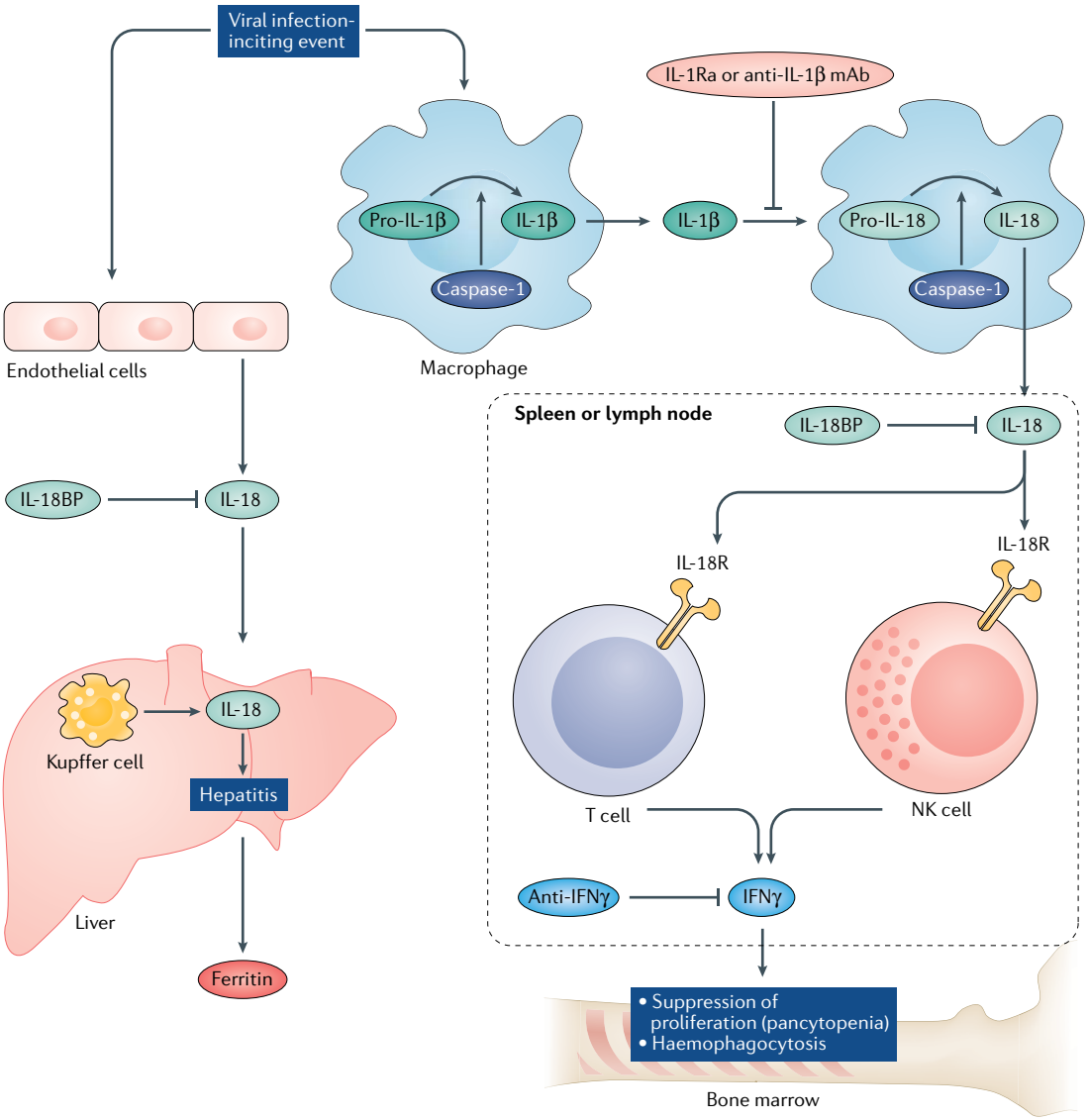


Fig. 5 | **IL-1 family members in macrophage activation syndrome.** Viral infections and predisposing conditions stimulate the synthesis of pro-IL-1β in macrophages, followed by caspase-1 cleavage, which results in the release of mature IL-1β. The released IL-1β can then stimulate the activation of caspase-1 and cleavage of pro-IL-18, which can occur in the same or in a different macrophage. IL-18 is then released. IL-18 binds to the IL-18 receptor (IL-18R, consisting of IL-1 receptor 5 (IL-1R5) and IL-1R7) on natural killer (NK) cells and T cells, which triggers the production and release of IFNγ. In the bone marrow, IFNγ suppresses haematopoiesis and activates bone marrow macrophages to perform haemophagocytosis, resulting in pancytopenia. An alternative mechanism for IL-18-mediated macrophage activation syndrome (MAS) is the release of IL-18 from mesenchymal cells such as endothelial cells. The exact mechanism by which pro-IL-18 is processed and mature IL-18 is released from endothelial cells is unclear. IL-18 binds to IL-18R in the liver and induces hepatitis via an increase in Fas ligand, causing the liver to release ferritin. The fixed macrophage pool in the liver, known as Kupffer cells, is also a source of IL-18. MAS can be treated by blocking IL-18 directly with IL-18 binding protein (IL-18BP), or by targeting IL-1β with IL-1 receptor antagonist (IL-1Ra) or anti-IL-1β monoclonal antibodies (mAb). Anti-IFNγ antibodies might also be effective in reducing the symptoms of MAS.

study, a substantial reduction in OA pain and improved joint function were reported by those treated with canakinumab compared with those who received placebo[109]. Patients who received 150 mg of canakinumab quarterly also had a low incidence of OA (1.12 per 100 person years, compared with 1.67 per 100 person years for placebo; $P < 0.001$)[109]. However, although canakinumab treatment was effective at reducing OA pain, no data exist as to whether canakinumab treatment also reduced cartilage loss.

Systemic use of antibodies neutralizing IL-1β (canakinumab) or blocking IL-1R1 (AMG108) seems to reduce pain in some patients with OA, particularly those with high Kellgren–Lawrence scores, but the risks, costs and inconsistent findings of such parenteral therapies limit their widespread use. In particular, systemic antibody treatment seems unlikely to be a popular therapy compared with orally active inhibitors that target IL-1β, such as NLRP3 inhibitors (reviewed in[198]). Oral inhibitors of NLRP3, such as dapansutrile[153], which reduces joint inflammation in murine models[199], have the potential to be effective treatments for OA; preliminary studies have reported reduced production of matrix metalloproteinases (MMPs) by chondrocytes following in vitro incubation with the NLRP3 inhibitor sulphoraphane[182,200]. Oral therapy with small-molecule NLRP3 inflammasome inhibitors could some day be a rational option for reducing pain; however, further clinical studies are required, and whether long-term use can prevent cartilage loss remains to be studied.

IL-33

IL-33, the third member of the IL-1 subfamily (FIG. 1a), was initially identified as the ligand for the then-orphan receptor IL-1R4 (REF.[16]). IL-33 first forms a heterodimer with IL-1R4, then a heterotrimeric complex with IL-1R3 (REFS[50,201,202]). Signal transduction via IL-1R4 promotes T_H2 cell responses and is the basis for the association of IL-33 with allergic diseases[203,204], although IL-33 is also associated with T_H1 cell responses[205]. Although pro-IL-33 contains a classic caspase-1 cleavage site, caspase-1 actually inactivates this cytokine[206]. Instead, processing occurs extracellularly via enzymes such as neutrophil elastase and cathepsin, which cut pro-IL-33 into increasingly active mature forms[27]. The release and extracellular processing of pro-IL-33 enables a rapid biological response that thus allows IL-33 to function as a pro-inflammatory alarmin. Similar to IL-1α, pro-IL-33 translocates to the nucleus[207], but its nuclear function remained unclear, until recently. In mice lacking the IL-33 nuclear localization sequence, IL-33 does not translocate to the nucleus and the mice develop lethal inflammation and large numbers of eosinophils in their organs[208]. Thus, nuclear sequestration of IL-33 might function to protect cells from inflammation. Overall, IL-33 seems to be involved in systemic responses to disease but also in local inflammation, such as occurs in mouse models of arthritis.

The fact that IL-33–IL-1R4 interactions trigger T_H2 cell responses means that some of the anti-inflammatory effects attributed to IL-33 in animal models of inflammation are potentially caused by T_H2 cell-related cytokines[209]. For example, in mice with CIA, treatment with recombinant IL-33 reduced joint inflammation, increased circulating concentrations of T_H2 cell-related cytokines, reduced IFNγ production and increased the number of eosinophils and type 2 innate lymphoid cells[210]. In another study, treatment with recombinant IL-33 also ameliorated CIA[211]. However, the results of studies in which mice are treated with recombinant IL-33 are dependent on the doses selected; some studies use doses that are orders of magnitude above in vivo concentrations. Indeed, data obtained using mice deficient for IL-33 have cast doubt on the putative pro-inflammatory role of recombinant IL-33 in CIA. IL-33 deficiency did not affect disease severity in mice with K/B×N serum transfer-induced arthritis[212], and in IL-33-deficient mice with CIA, T_H1 cell and T_H17 cell responses were not suppressed and no differences were seen in disease severity between the IL-33-deficient mice and wild-type mice[213]. A similar lack of differences between IL-33-deficient mice and wild-type mice was also noted in a model of psoriasis[213].

Although several studies support the concept that IL-33 is involved in RA, this evidence primarily comes from correlations between disease severity and the presence of IL-33 in the synovium, synovial fluid or circulation[214–217], and ex vivo culture experiments. For example, when cultured synovial fibroblasts from patients with RA are exposed to TNF, the gene expression and synthesis of IL-33 increase and feed back to augment the ability of TNF to induce the production of MMP1 and MMP3 by these cells[218], which could contribute to cartilage loss in arthritic joints. IL-33-induced activation of mast cells is also relevant to the biology of RA. IL-33 stimulates the release of histamine, chemokines and anti-inflammatory cytokines by synovial mast cells, which can inhibit monocyte activation[219]. Although antibodies specific for IL-33 and IL-1R4 are in clinical trials for allergic diseases such as asthma and contact dermatitis[220,] patients with RA have not yet been treated with IL-33-blocking therapies. Therefore, it remains unclear whether circulating IL-33 can be used as a biomarker for RA or whether IL-33 contributes to pathological processes in RA. However, soluble IL-1R4 and IL-33 have potential as biomarkers of cardiovascular disease activity in patients with RA[214].

Overall, IL-33 might have a pro-inflammatory role in rheumatic diseases, but could also be involved in an anti-inflammatory mechanism by skewing immune reactions towards T_H2 cell-mediated responses. IL-33-induced mast cell activation potentially contributes to RA, and antibodies in clinical trials for asthma and other allergic diseases could be used to verify the role of IL-33 in RA pathogenesis.

IL-18 subfamily

IL-18

Similar to IL-1β, IL-18 is first synthesized as an inactive precursor without a signal peptide and remains an intracellular cytokine until it is processed by caspase-1 and released; inhibition of the NLRP3 inflammasome reduces caspase-1-mediated processing of pro-IL-18. However, similar to IL-1α, pro-IL-18 is constitutively present in mesenchymal cells, such as endothelial

cells, in the epithelial cells of the entire gastrointestinal tract, in keratinocytes and in the brain (reviewed elsewhere[221,222]). Also similar to pro-IL-1α, pro-IL-18 is released during necrosis[223]. Although IL-18 was first identified for its IFNγ-inducing properties[15,224], it has several other properties and functions in local and systemic inflammatory diseases such as MAS[221,222]. Also present in disease is IL-18BP (tadekinig alfa), a naturally occurring 20-kDa glycoprotein antagonist of IL-18 that was discovered in 1999 (REF.[225]). In healthy individuals, circulating concentrations of IL-18BP are 2–3 ng/ml (REF.[226]), which is a 10–20-fold molar excess over concentrations of circulating IL-18. IL-18BP binds and neutralizes IL-18 with an unusually high affinity of 0.4 nM (REF.[225]) and a dissociation coefficient that might be as low as 0.05 nM (REF.[227]). The high affinity of IL-18BP for IL-18 means that most circulating IL-18 is bound to IL-18BP and is therefore inactive. Concentrations of free IL-18 can be calculated from total IL-18 concentrations[226] or a specific enzyme-linked immunosorbent assay (ELISA) for free IL-18 can be used[227], and the amount of free IL-18 correlates with disease severity in sepsis[226], SLE[228], granulomatosis with polyangitis[229], Crohn's disease[230], AoSD[227] and MAS[231]. IL-18BP has been used to treat diseases such as NLRC4-associated hyperinflammation[232] and AoSD[176], and in an ever-expanding number of clinical trials, but is not presently approved for use in any rheumatic diseases.

IL-18 in macrophage activation syndrome. MAS, also known as secondary haemophagocytic lymphohistiocytosis, presents as a severe hyper-inflammatory state with pancytopenia, liver dysfunction, increased ferritin and coagulopathy[233–237]. The development of MAS is associated with infectious agents, such as Epstein–Barr virus, cytomegalovirus, herpesvirus, intracellular bacteria and parasites, and with lymphomas, especially those of the T cell lineage (as reviewed elsewhere[222]). Patients with sJIA or AoSD are at high risk of developing MAS, which can be life-threatening. MAS can also occur in SLE, Kawasaki disease and systemic vasculitis[234–237], and plasma concentrations of IL-18 in patients with MAS are 20–30-fold higher than in patients with RA[227,231,238–240]. The inciting event in MAS often takes place in an individual who is predisposed to increased cytokine production, for example, in a 'trained immunity' setting in which macrophages produce IL-1β. Mechanistic studies have implicated IFNγ as important in the thrombocytopenia and immunological abnormalities that occur in this disorder[222,231] (FIG. 5). For example, high amounts of free IL-18 cause T cells and NK cells to release IFNγ, which suppresses the bone marrow and manifests as pancytopenia[241,242]. Another event that probably occurs in MAS is the release of IL-18 from the gut and endothelium, and in the liver, IL-18 induces the production of Fas ligand, hepatic cell death and increased ferritin release[223,244]. In fact, high plasma concentrations of ferritin can be used to distinguish clinical MAS from a disease flare in patients with sJIA[233,238,243–246].

Patients with a gain-of-function mutation in *NLRC4* (REF.[232]) or deficiency in X-linked inhibitor of apoptosis protein (XIAP)[247] experience a life-threatening hyper-inflammatory state with high levels of free IL-18 that is similar to MAS; treatment of these patients with IL-18BP provides resolution of the inflammatory state[232,238,248–251]. IL-18BP has also been used effectively to treat patients with refractory AoSD[177,227]. Treatment with anakinra is effective for patients with sJIA or AoSD who develop MAS[252,253] and the mechanism includes a reduction in the processing of pro-IL-18 into an active cytokine[254] (FIG. 5). Importantly, MAS can masquerade as septic shock, and some patients enrolled in anakinra trials for the treatment of septic shock were retrospectively identified as having MAS[255,256]. In a re-analysis of the data from one of these trials[256], those patients identified as having MAS had an all-cause 28-day survival of 65% when treated with anakinra, compared with 35% in those treated with placebo ($P = 0.007$)[257]. Overall, although treatment with canakinumab or anakinra is effective for MAS, neutralization of IL-18 with IL-18BP could hasten the resolution of the hyper-inflammatory state.

IL-18 in rheumatic diseases. Being constitutively present in nearly all organs, IL-18 is likely to contribute to many diseases. Numerous studies have examined associations between *IL18* polymorphisms and RA risk, but a meta-analysis revealed no consistent association[258]. In a study of 90 patients with RA, circulating concentrations of IL-18 were modestly elevated compared with those of healthy individuals and were greater in those with erosive disease than in those with non-erosive disease[259]. Concentrations of IL-18 in the synovial fluid were also higher in patients with erosive disease than in those with non-erosive disease[259]. Synovial tissue from patients with RA also had increased expression of *IL18* and *IL1R5* compared with synovium from patients with OA[260]. In primary human synovial cell cultures, TNF and IL-1β induce IL-18 production[260], and a similar induction of plasma IL-18 by exogenous IL-1β has been seen in mice[254]. A phase I trial of IL-18BP for RA was carried out, but the drug was discontinued for this indication by the manufacturer[261]. Overall, the evidence for a role for IL-18 in RA is not convincing.

By contrast, a role does exist for IL-18 in SLE. A 10-year follow-up study in 96 patients with paediatric-onset SLE concluded that, more than any other cytokine, serum concentrations of IL-18 correlated with global disease activity, flares of renal disease and predicted long-term outcome in these patients[262]. A study in adults also found an increase in circulating IL-18 in patients with active SLE that was associated with decreased renal function[263]. Concentrations of free IL-18 are elevated in patients with active SLE in comparison with healthy volunteers or patients with inactive disease[228,264,265] and correlate positively with platelet count and concentration of anti-double-stranded DNA antibodies and urinary protein, but negatively with serum complement C3 (REF.[266]). Increased concentrations of IL-18 have also been found in patients with subacute cutaneous lupus erythematosus[267]. The results of these studies[228,264,265] and the relationship between IL-18 and IFNγ, which is also important in SLE, suggest that a rationale exists to trial IL-18BP for patients with active SLE.

IL-37

IL-37 has a unique role in the IL-1 family, as this cytokine broadly suppresses innate inflammation as well as acquired immune responses. Following its discovery in silico in 2000 (REF.[17]), the results of initial studies on IL-37 were confusing because this cytokine bound to IL-1R5, which is the receptor for IL-18 (FIG. 1b), and thus seemed to function as an antagonist for IL-18 (REFS[268,269]). Subsequent studies revealed that IL-37 is not a direct antagonist for IL-18, but is instead a broad inhibitor of innate immune responses[19,20,270]. IL-37 binds to IL-1R5 and a heterotrimeric complex is then thought to form with the co-receptor IL-1R8, which orchestrates several intracellular mechanisms that inhibit innate immune responses[38,271]. In general, the anti-inflammatory properties of IL-37 are not observed in the absence of IL-1R8; however, increased NK cell antitumour activity does occur in the absence of IL-1R8 (REF.[272]), suggesting that IL-37 and IL-1R8 are immune checkpoints.

IL-37 is a suppressor of innate immune responses[270]. Because IL-37 suppresses mTOR while at the same time increasing the phosphorylation of AMP kinase[270], it can be considered an endogenous form of rapamycin. This decrease in mTOR and increase in AMP kinase activity by IL-37 represents a reversal of the Warburg effect (the use of glycolysis to generate ATP). Most inflammatory and immune processes take place at a metabolic cost to the host. Recombinant human IL-37 administered to mice blunts the metabolic cost of inflammation by increasing oxidative phosphorylation and reducing inflammatory succinate[273]. Similar changes take place in mononuclear cells such as macrophages and dendritic cells.

IL-37 also inhibits acquired immune responses by tolerizing dendritic cells[274]. In that study, maturation of dendritic cells was arrested in transgenic mice expressing human IL-37 and the dendritic cells produced high levels of IL-10, which contributes to tolerization. In addition to decreased mature dendritic cells, IL-37 also suppressed expression of MHC class II. The broad anti-inflammatory properties of IL-37 are consistent with a primary systemic role, although IL-37 can also function at a local tissue level, such as in adipose tissue[275,276].

A role for IL-37 has been examined in mouse models of inflammatory arthritis. Recombinant human IL-37 administered systemically to mice suppressed joint inflammation in a model of inflammation induced by intra-articular injection of streptococcal cell wall fragments[39]. However, no reduction in inflammation was seen in similarly treated mice deficient in IL-1R8 (REF.[39]). In this model, the 52% reduction in joint inflammation was associated with a statistically significant reduction in synovial IL-1β, IL-6, TNF, CCL2 and myeloid peroxidase[94].

Missense coding mutations in cytokines are rare compared with missense coding mutations in their receptors, intracellular proteins and kinases. There are several missense mutations in *IL37* associated with disease risk. For example, the SNP rs3811047 (change from A to G in exon 2) results in a threonine to alanine change at position 42 in IL-37 (REF.[277]). In Han Chinese patients with RA, this SNP is associated with reduced joint disease scores and less pain compared with those without the mutation, suggesting that this SNP might be gain-of-function for the anti-inflammatory properties of IL-37 (REFS[277,278]). According to data from the 1000 Genomes Project, a major variant of *IL37* that includes five non-synonymous SNPs and has a penetrance of 16% in Africa and 7% worldwide is likely to encode a dysfunctional protein[279]. Individuals with this variant might have decreased amounts of IL-37, which could contribute to more pronounced inflammation[279].

Several studies have reported high concentrations of circulating IL-37 in patients with RA[280–285], sJIA[286,287] or AoSD[288] and an association between increased IL-37 and T cell activation in patients with RA[289,290]. In PBMCs from patients with sJIA, recombinant IL-37 suppresses the production of IL-6, IL-17 and TNF[286]. Relevant to the function of IL-37, natural and recombinant IL-37 form spontaneous homodimers that are routinely seen in western blots of stimulated human PBMCs[270] and recombinant forms of IL-37 (REFS[269,291,292]), respectively. These homodimers seem to function to reduce IL-37 anti-inflammatory activity, probably by preventing the binding of IL-37 to IL-1R5, and result in a bell-shaped dose–response curve[291,293]. Single amino acid changes in recombinant IL-37 prevent the formation of homodimers and improve the suppressive capacity of IL-37 in vitro[291,292]. Preventing IL-37 homodimer formation, therefore, represents a potential avenue to pursue to develop IL-37 therapeutically[291]. Compared with IL-37 concentrations in healthy individuals, IL-37 is also increased in patients with ankylosing spondylitis[293], psoriasis[294] and SLE[295]. These and similar studies support the concept that as inflammation increases, IL-37 expression increases to function as an appropriate response to limit disease severity. By contrast, in Behçet disease[296,297], calcific aortic valve disease[41], asthma[40,298], obesity with insulin resistance[276], periodontal disease[299], allergic rhinitis[300], alcoholic liver disease[301] and non-small-cell lung cancer[302], amounts of IL-37 mRNA and protein are lower than in tissues from healthy individuals, suggesting a relative deficiency. An important concept to emerge from such studies is that reduced amounts of endogenous IL-37 seem to contribute to the severity of inflammation in these diseases.

Overall, a clear role seems to exist for IL-37 in rheumatic diseases such as sJIA, AoSD and RA, in which polymorphisms are associated with disease severity. The suppression of inflammation in mouse models of several human diseases by recombinant human IL-37 suggests that IL-37 could be developed and tested as a treatment for rheumatic and non-rheumatic diseases.

IL-36 subfamily

IL-36 cytokines

IL-36α, IL-36β, IL-36γ and IL-36Ra were each first reported as individual genes between 2000 and 2002 as a result of in-silico studies[17,18,303,304]. IL-36 cytokines and IL-36Ra are mostly found in the skin, where they participate in epidermal cornification[305] and to date, 11 missense mutations in *IL36RN*, which encodes IL-36Ra, have been discovered that are associated with an increased risk of pustular psoriasis[306–308]. IL-36Ra specifically binds to IL-1R6 and functions as a true receptor antagonist, thereby preventing IL-36 cytokine-mediated signalling (FIG. 1c). IL-36α, IL-36β and IL-36γ exist as inactive

precursors in epithelial cells that must be processed to produce biologically active cytokines; a similar process is required for fully active IL-36Ra[24]. This processing to produce active molecules is thought to require neutrophil elastase, as inhibition of neutrophil elastase prevents IL-36 activity[309,310]. In general, IL-36 cytokines function primarily in skin diseases and at present, no data exist to support a role for these cytokines in systemic disease.

IL-1R6 is constitutively expressed by dendritic cells and $CD4^+$ T cells[311], suggesting that IL-36 cytokines are potentially immunologically active. For example, IL-36α, IL-36β and IL-36γ are each able to induce IL-2, IL-1β, IL-12 and IL-17 production, and the expression of CD80, CD86 and MHC class II molecules on dendritic cells[311]. The function of IL-36 has been studied in mouse models of skin hypersensitivity, lung inflammation, inflammatory bowel disease, kidney disease, type I diabetes mellitus and RA (as reviewed elsewhere[312–315]), although studies in models of RA have been few[316,317]. In mice with CIA, expression of IL-36 cytokines and IL-36Ra was increased in joint tissue compared with mice without disease[316]. Expression of IL-36 cytokines and IL-36Ra was also high in synovium from patients with RA and correlated positively with IL-1β expression, but not with IL-17 expression[316]. In comparison with psoriasis, IL-36 cytokines are probably not important in joint diseases; <30% of synovia from patients with RA were positive for IL-36 cytokines by PCR, ELISA and immunohistochemistry[316]. Nevertheless, the low ratio of IL-36 agonists to IL-36Ra[316] favours an agonistic role for IL-36 cytokines in RA. High concentrations of IL-36α in the circulation and salivary glands have also been found in patients with primary Sjögren syndrome[318].

Overall, most investigations into IL-36 cytokines have focused on the agonistic role of IL-36 in psoriasis and the available studies of IL-36 in RA in humans and in animal models do not support a substantial contribution of IL-36 cytokines in this disease. However, IL-36Ra could potentially be developed and studied in clinical trials for rheumatic diseases, particularly PsA[319].

IL-38

IL-38 is the most recently added member of the IL-36 subfamily (FIG. 1c). However, whereas the binding of IL-36 cytokines to IL-1R6 transmits a pro-inflammatory signal, the binding of IL-38 to IL-1R6 results in the suppression of inflammation[21,320]. For example, recombinant human IL-38 inhibits the production of IL-17 and IL-22 by human PBMCs in vitro[320]. The anti-inflammatory properties of IL-38 have been proposed to be caused by the recruitment of IL-1R9 following the binding of IL-38 to IL-1R6, the formation of a trimeric signalling complex and the triggering of downstream anti-inflammatory signalling pathways[321]. However, the formation of a trimeric structure among IL-1R6, IL-38 and IL-1R9 has not yet been demonstrated. As is the case with IL-37, IL-38 is emerging as an anti-inflammatory member of the IL-1 family[21]. In a large GWAS study that included 80,000 participants at high risk of a cardiovascular event, *IL38* was associated with high CRP concentrations[322]. This newest member of the IL-1 family might function systemically, as elevated circulating levels of IL-38 are associated with various inflammatory diseases[150,285,323–327]. Interestingly, and uniquely in cytokine biology, IL-1R9, considered the co-receptor for IL-38, is encoded on the X-chromosome[30,328], and so might have a role in the sex bias that occurs in several rheumatic diseases.

Although IL-38 is present in synovial tissues from patients with RA[329], and correlates with the expression of macrophage colony-stimulating factor, IL-1β and chemokines[316], IL-38 seems to be a B cell product, as this cytokine is found in tonsils and proliferating B cells[330,331]. Concentrations of circulating IL-38 are increased in patients with RA and correlate with the expression of the other members of the IL-36 subfamily[150]. In antibody-induced arthritis, compared with wild-type mice, those mice deficient in IL-38 had a high degree of joint inflammation, which was associated with increased synovial expression of IL-1β and IL-6 (REF.[329]). In three different models of RA, adenovirus-induced overexpression of IL-38 in the joints of mice reduced the severity of disease compared with joints injected with the control virus[317]. These decreases in disease severity correlated with decreased amounts of IL-17, IL-23 and IL-22 (REF.[317]). Systemic treatment of mice with recombinant human IL-38 also reduced disease in mice with streptococcal cell wall-induced knee arthritis and reduced joint inflammation in mice following intra-articular administration of MSU crystals[332].

In addition to RA, concentrations of circulating IL-38 are also increased in patients with SLE compared with healthy individuals[327]. In this study[327], only 16% of 372 serum samples had measurable IL-38 (range 60–5,900 pg/ml) and of these, the concentrations of IL-38 in serum from patients with SLE with active disease were 11-fold higher than in those with inactive disease. In these patients, high IL-38 concentrations correlated with an increased risk of renal disease and of active disease[327]. Furthermore, in PBMCs transfected with siRNA that targets IL-38, the production of pro-inflammatory cytokines increased 28-fold over cells transfected with control siRNA[327]. A similar increase in the production of pro-inflammatory cytokines occurred when PBMCs were transfected with IL-37-targeting siRNA[270]. In lupus-prone MRL/lpr mice, treatment with recombinant human IL-38 reduced several disease manifestations, including proteinuria and skin lesions, and also reduced circulating IL-17 and IL-22 (REF.[333]).

IL-38 is now established as an anti-inflammatory cytokine with properties similar to those of IL-37. Like IL-37, IL-38 suppresses T cell activation and therefore might function not only in RA, but also in plaque psoriasis[323]. A therapeutic role for recombinant IL-38 is likely to be considered in the future. Because the co-receptor for IL-38 is on the X chromosome, IL-38-based therapies might be particularly suited to women with autoimmune diseases such as SLE, RA or psoriasis[323].

Targeting IL-1 family members

IL-1α and IL-1β

As shown in TABLE 1, there are three approved biologic drugs that reduce the activity of IL-1α and/or IL-1β, anakinra, rilonacept and canakinumab, and several that are in clinical trials. Anakinra, the recombinant form of

Table 1 | **Treatments for rheumatic diseases that target IL-1 family members**

Drug name	Target	Type of agent	Indication(s)	Refs
Approved[a]				
Anakinra	IL-1R1 (IL-1α and IL-1β)	Recombinant human IL-1Ra	CAPS[a], RA[a], AoSD, sJIA, gout and many other off-label indications	Reviewed in[14,361]
Rilonacept	IL-1β, IL-1α and IL-1Ra	IL-1R1 fusion protein	CAPS[a], AoSD	362,363
Canakinumab	IL-1β	Anti-IL-1β mAb	AoSD[a], CAPS[a], FMF[a], gout[a], sJIA[a]	66,347,364
In clinical trials				
Gevokizumab	IL-1β	Anti-IL-1β mAb	Behçet disease	349
MABp1	IL-1α	Anti-IL-1α mAb	Hidradenitis suppurativa	90
AMG 108	IL-1R1 (IL-1α and IL-1β)	Anti-IL-1R1 mAb	OA, RA	197,350
Lutikizumab	IL-1α and IL-1β	mAb with dual affinity for IL-1α and IL-1β	OA	192,193,351,365
Tadekinig alfa	IL-18	Recombinant human IL-18BP	AoSD	177,227

AoSD, adult-onset Still's disease; CAPS, cryopyrin-associated periodic syndromes; FMF, familial Mediterranean fever; IL-1R, IL-1 receptor; IL-1Ra, IL-1 receptor antagonist; IL-18BP, IL-18 binding protein; mAb, monoclonal antibody; OA, osteoarthritis; RA, rheumatoid arthritis; sJIA, systemic-onset juvenile idiopathic arthritis. [a]Information about approvals for the listed indications is detailed in Supplementary Table S2.

IL-1Ra, blocks both IL-1α and IL-1β and is approved for the treatment of CAPS in the EU, the USA and several other countries (see Supplementary Table S2). In the USA, anakinra is mostly used for off-label indications, particularly to treat acute flares of gout. In France, there is also off-label use of anakinra for gout, and also for FMF and hyper IgD syndrome[334]. In Italy, a study in 475 adults and children revealed off-label anakinra use in 80% of the studied cohort for the treatment of 37 different indications[335]. Off-label use occurred more frequently with anakinra than with canakinumab ($P<0.001$)[335]. In patients with gout and type 2 diabetes, off-label use of anakinra resolved the gout as well as the diabetes[336]. In RA, comorbidities such as heart failure reduce the quality of life for patients; anakinra was used to treat both conditions[337]. In an observational study of patients with RA and type 2 diabetes mellitus, both the arthritis and haemoglobin A1C, a measure of the average blood glucose levels during a 3-month interval, were reduced by anakinra[338]. Daily anakinra use was also compared with standard-of-care treatment using TNF inhibitors in a multicentre, randomized, prospectively controlled trial in patients with both RA and type 2 diabetes[339]. After 3 and 6 months of treatment, haemoglobin A1C was significantly reduced in patients treated with anakinra but not in patients treated with TNF inhibitors ($P<0.001$), although reductions in arthritis scores were similar for both treatments[339]. These studies treating comorbidities are consistent with the broad anti-inflammatory mechanisms of blocking IL-1 in more than a single indication with a single intervention.

The second biologic drug, rilonacept, is an Fc fusion protein comprising the extracellular domains of IL-1R1 and IL-1R3, which functions as a soluble receptor[340]. Rilonacept is currently approved for the treatment of CAPS in the USA (see Supplementary Table S2). The third biologic drug, canakinumab, an anti-IL-1β monoclonal antibody, is used off-label for autoinflammatory diseases such as FMF[313–343] and Schnitzler syndrome[334,344–346], as well as to treat RA[347]. Canakinumab is approved in Europe and the USA for use in several autoinflammatory diseases (such as TNF receptor-associated periodic syndrome, CAPS, FMF and hyper IgD syndrome; see Supplementary Table S2). Treating RA[347] or other rheumatic diseases with canakinumab has the advantage of treating the primary disease as well as comorbidities. In the CANTOS trial, canakinumab administration four times a year for 4 years reduced fatal and non-fatal myocardial infarctions, hospitalizations for urgent re-vascularization, heart failure, gout, type 2 diabetes mellitus and cancer[109,117,118,348], which are well-known comorbidities in patients with RA.

Another monoclonal antibody targeting IL-1β is gevokizumab, which has been tested for use in the treatment of uveitis in patients with Behçet disease[349] and for the treatment of type 2 diabetes[114]. In Behçet disease, parenteral gevokizumab resolved the uveitis rapidly and restored sight[349]. A human monoclonal antibody that targets IL-1R1, AMG108, has been tested in randomized, placebo-controlled trials in OA[197] and RA[350]. AMG108 is unlikely to be developed further for OA because of limited efficacy, but the antibody could be developed for the same indications as anakinra, such as autoinflammatory diseases. The dual antibody lutikizumab is capable of binding two molecules of IL-1α and two molecules of IL-1β simultaneously[351] and has been tested for hand OA[192]; however, as mentioned above, the trial did not meet its primary end point. One concludes that antibodies to IL-1α, IL-1β or IL-1R1 do not result in robust responses in OA. One explanation is because of limited access of antibodies to the joints and particularly chondrocytes. OA pain was significantly decreased in the CANTOS trial, demonstrating efficacy; however, the trial lasted 4 years, whereas trials for OA last less than 1 year. As discussed above, small molecules such as oral NLRP3 inhibitors that target IL-1β are likely to be tested in OA.

The results of the CANTOS trial[109] demonstrated that targeting IL-1β with canakinumab can reduce

inflammation as well as cardiovascular events. Whether low-dose methotrexate, which is used to treat RA and several other rheumatic diseases, could produce similar benefits was tested in a randomized, double-blind, placebo-controlled trial in 4,786 patients with a previous infarction or multi-vessel disease and type 2 diabetes mellitus[352]. However, the trial was stopped after 2.3 years because there was no difference between those treated with methotrexate and the placebo arm. Methotrexate also failed to lower CRP, IL-6 and IL-1β[352]. One can conclude that methotrexate does not protect against atherosclerosis.

Other IL-1 family members

Presently, there are no approved treatments that specifically reduce the activity of IL-18. However, IL-18BP has a high affinity for IL-18 (REF.[225]), making it an excellent candidate for treating IL-18-mediated rheumatic diseases. A phase I study[353] in which IL-18BP was administered to a small number of patients with RA or psoriasis has been performed, but the data have not been published and no further studies have been carried out for these indications. Nevertheless, IL-18BP has emerged as a treatment for reducing the activities of IL-18 in AoSD[177,227]. IL-18BP is presently not approved but is in clinical trials for genetic forms of MAS[354].

An anti-IL-1R4 antibody, CNTO 7160, which blocks IL-33, is currently in clinical trials for the T_H2 cell-mediated diseases asthma and atopic dermatitis[355,356]. However, reducing T_H2 cell-mediated responses with IL-33 blockade could worsen some rheumatic diseases owing to a shift away from the protective role of T_H2 towards T_H1 cell-mediated responses. For example, in a mouse model of RA, recombinant IL-33 reduced inflammation, which was associated with increased T_H2 responses. Whether anti-IL-33 will worsen RA remains unknown. Similarly, a blocking antibody to the co-receptor IL-1R3 is being developed that will reduce the activity of IL-1α, IL-1β, IL-33, IL-36α, IL-36β and IL-36γ[49]. In murine models of gout, asthma and psoriasis, blocking IL-1R3 has been effective compared with IL-1R1 blockade. One rationale for using anti-IL-1R3 in human autoimmune diseases is based on the specific blockade of IL-33 and IL-36 cytokines via the ligand binding receptor and the benefit of reduced inflammation by blocking IL-1α and IL-1β at the same time. Preclinical studies with IL-36Ra have also been carried out with a focus on psoriasis[24,357] and, if developed, IL-36Ra would probably be evaluated for that disease.

A different approach would be to promote anti-inflammatory responses in rheumatic diseases, which makes recombinant IL-37 an attractive therapeutic candidate. Although only preclinical studies in mice have been performed to date with recombinant IL-37 (reviewed elsewhere[19]), the potential suppression of innate inflammation responses by IL-37 would make it a good candidate for use in RA, gout and for several comorbidities. Recombinant IL-38 would probably have similar targets to recombinant IL-37. The future of the IL-1 family members in treating diseases continues to expand from the early use of recombinant IL-1Ra (anakinra). Now there are validated trial data with specific neutralization of IL-1β, IL-1α, IL-33 and IL-18. IL-37 and IL-38 are the latest cytokines to be studied in preclinical models based on the new area of broadly suppressing innate inflammation. The use of IL-1R3 blockade and reduction of the activities of six members of the IL-1 family with one antibody are also new.

Conclusions

The 11 members of the IL-1 family are present locally and/or systemically in individuals with rheumatic diseases and can contribute to pathogenesis in either a pro-inflammatory or anti-inflammatory manner. In particular, the roles of IL-1β, IL-1α and IL-18 have been validated in clinical trials of specific inhibitors in several diseases; for example, blocking IL-1β in auto-inflammatory diseases, blocking IL-1α in hidradenitis suppurativa and blocking IL-18 in AoSD. Targeting IL-1β-mediated and IL-18-mediated diseases by means of oral NLRP3 inhibitors is currently being studied, and could reduce the use of parenteral biologics such as anakinra, canakinumab and IL-18BP. The roles of other IL-1 family members, such as IL-33, IL-36α, IL-36β, IL-36γ, IL-37 and IL-38, in rheumatic diseases, and their potential usefulness as therapies or therapeutic targets, are still being investigated in preclinical studies. In the future, recombinant forms of the newer members of the IL-1 family should be developed for use in clinical trials; for example, IL-36Ra should be developed to treat IL-36-mediated skin diseases, and recombinant forms of IL-37 or IL-38 could be developed to non-specifically target innate inflammation. Overall, although much has been accomplished by studying the IL-1 family, more remains to be discovered.

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Competing interests
C.A.D. serves as chair of the SAB of Olatec Therapeutics, LLC, which develops the NLRP3 inhibitor OLT1177 (Dapansutrile).

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