Nature Reviews Rheumatology | Published online 22 Sep 2017; doi:10.1038/nrrheum.2017.154

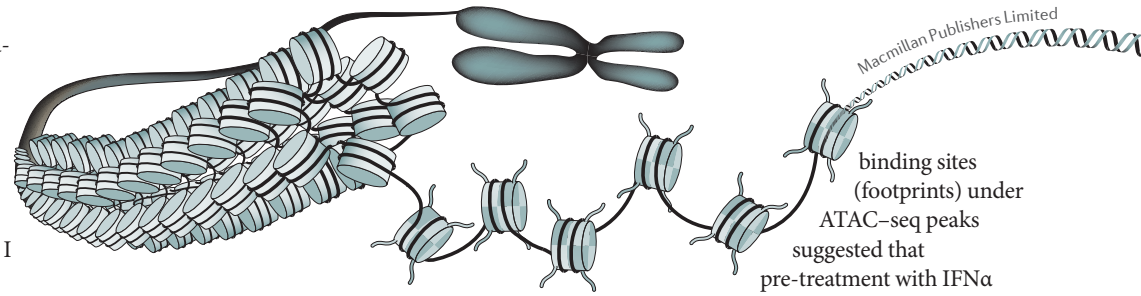
INFLAMMATION

Cytokines alter inflammatory responses via chromatin changes

TNF-induced 'cross-tolerance' has been reported to limit the inflammatory response to Toll-like receptor (TLR) ligands, but how this inhibitory mechanism is overridden to enable TNF to drive chronic inflammation is not well understood. New research published in *Nature Immunology* reveals that type I interferons effectively abolish TNF-induced tolerance by reprogramming the macrophage epigenome and thus altering the response to inflammatory stimuli.

"We discovered a new function for type I interferons in reversing tolerization of inflammatory genes by TNF," reports corresponding author Lionel Ivashkiv. "The mechanism was [the] opening of chromatin, which enabled robust transcriptional responses to very weak upstream signals," he continues. "These results suggest a way by which interferons promote chronic inflammation by potentiating and extending the inflammatory functions of TNF."

Transcriptomic analysis using RNA sequencing revealed that pre-treatment with TNF substantially altered the response of primary human macrophages to subsequent LPS-mediated stimulation of TLR4. The transcription of a number LPS-inducible genes encoding pro-inflammatory molecules and NFκB-dependent genes was inhibited by TNF treatment, indicating tolerization, whereas the expression of cytokine-induced genes was enhanced. Pre-treatment with both IFNα and TNF abolished TNF-mediated tolerance, resulting in increased LPS-induced gene expression. Notably, pre-treatment with IFNα alone did not alter LPS inducibility, suggesting a role for crosstalk between TNF and interferons.



> "type I interferons effectively abolish TNF-induced tolerance"

To investigate the role of epigenetic mechanisms in the regulation of TLR4 responses by TNF, the researchers analysed chromatin accessibility and positive histone marks associated with open chromatin using a gemone-wide approach involving chromatin immunoprecipitation followed by deep-sequencing (ChIP–seq) and assay for transposase accessible chromatin with high-throughput sequencing (ATAC–seq). They found that treatment of macrophages with the combination of IFNα and TNF increased chromatin accessibility at the promoters of genes encoding inflammatory molecules, enabling strong transcriptional responses even to weak LPS signals.

"We performed to our knowledge the first 'digital footprinting in accessible chromatin' (under ATAC-seq peaks) in the immune system, which enabled identification of transcriptional networks that regulate gene expression," reports Ivashkiv. Analysis of occupied transcription factor binding sites (footprints) under ATAC–seq peaks suggested that pre-treatment with IFNα and TNF primes chromatin by co-operatively recruiting TNF-induced NFκB and interferon-induced transcription factors to the promoters of LPS-inducible genes.

Demonstrating the consequences of chromatin priming, IL-10 could only partially suppress LPS-induced expression of *IL6* in macrophages treated with TNF and IFNα. By contrast, IL-10 potently suppressed *IL6* expression in naive, LPS-stimulated macrophages.

Future work might explore the potential of targeting chromatin regulators in the context of rheumatic disease. "Aspects of the gene and chromatin regulation we discovered were mirrored in synovial macrophages from patients with rheumatoid arthritis and monocytes from patients with systemic lupus erythematosus," Ivashkiv recounts. "This suggests that these pathways can be therapeutically targeted to rebalance the inflammatory response to prevent toxicity while preserving host defense."

Sarah Onuora

ORIGINAL ARTICLE Park, S. H. *et al.* Type I interferons and the cytokine TNF cooperatively reprogram the macrophage epigenome to promote inflammatory activation. *Nat. Immunol.* http://dx.doi.org/10.1038/ni.3818 (2017)

Nature Reviews Rheumatology | Published online 14 Sep 2017

IN BRIEF

RISK FACTORS

Oral contraceptive use linked to lower risk of RA

In the population-based Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study, women with a history of oral contraceptive (OC) use had a decreased risk of developing anti-citrullinated protein antibody (ACPA)-positive rheumatoid arthritis (RA) compared with women who had never used OCs (OR 0.87, 95%CI 0.78–0.97). The risk of ACPA-positive RA among smokers was higher in those who had never used OCs than in those who had. The study could not confirm an association between breastfeeding and a decreased risk of either ACPA-positive or ACPA-negative RA.

ORIGINAL ARTICLE Orellana, C. *et al.* Oral contraceptives, breastfeeding and the risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211620 (2017)

CLINICAL TRIALS

Intravenous golimumab effective for PsA

Results of the phase III, randomized, double-blind GO-VIBRANT study show that patients with psoriatic arthritis (PsA) treated with intravenous golimumab 2 mg/kg (n = 241) had greater improvements in the signs and symptoms of the disease at week 14 and less radiographic progression at week 24 than those who received intraveous placebo (n = 239). 75.1% of those in the golimumab arm achieved ≥20% improvement according to ACR criteria (ACR20) at week 14, compared with 21.8% of the placebo group.

ORIGINAL ARTICLE Kavanaugh, A. *et al.* Safety and efficacy of intravenous golimumab in patients with active psoriatic arthritis: results through week 24 of the GO-VIBRANT study. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40226 (2017)

INFECTION

Infection risk after switching biologics

Patients with rheumatoid arthritis (RA) that fails to respond to treatment with a first TNF inhibitor have a similar risk of serious infection whether they switch to another TNF inibitor or to rituximab, according to an analysis of data from a UK registry. In the first year after switching, serious infection occurred in 164 (4.8%) of 3,419 patients treated with a second TNF inhibitor and in 81 (5.8%) of 1,396 patients treated with rituximab. The most common sites of serious infection in both groups were the lower respiratory tract and urinary tract, consistent with previous findings.

ORIGINAL ARTICLE Silva-Fernández, L. *et al.* Serious infection risk after 1 year between patients with rheumatoid arthritis treated with rituximab or with a second TNFi after initial TNFi failure: results from The British Society for Rheumatology Biologics Register for Rheumatoid Arthritis. *Rheumatology (Oxford)* https://doi.org/10.1093/rheumatology/kex304 (2017)

PAIN

Caution needed in use of gabapentinoids for LBP

A systematic review and meta-analysis of randomized controlled trials (RCTs) reveals only limited, low-quality evidence to support the use of pregabalin or gabapentin for the treatment of chronic low back pain (LBP). Results of the eight RCTs identified showed that gabapentin had a minimal benefit over placebo, and that pregabalin was inferior to other analgesics in relieving pain in adult patients with LBP of >3 months' duration. Use of the gabapentinoids was also associated with an increased risk of adverse events.

ORIGINAL ARTICLE Shanthanna, H. *et al.* Benefits and safety of gabapentinoids in chronic low back pain: a systematic review and meta-analysis of randomized controlled trials. *PLoS Med.* **14**, e1002369 (2017)

Nature Reviews Rheumatology | Published online 31 Aug 2017; doi:10.1038/nrrheum.2017.149

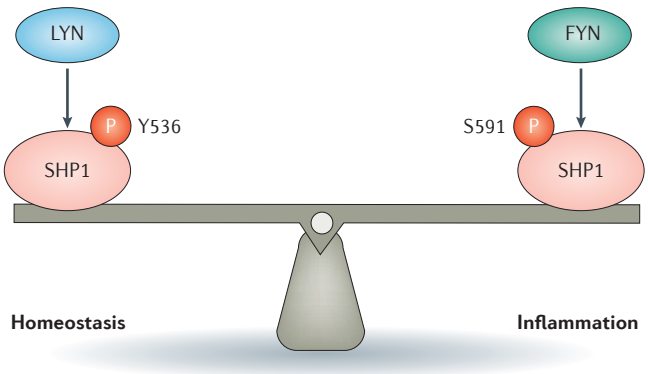
IMMUNOLOGY

Balancing immunoreceptor signalling

The Src-family kinases LYN and FYN differentially regulate immunoreceptor signalling by directing the phosphorylation of SH2 domain-containing protein tyrosine phosphatase 1 (SHP1, also known as PTPN6) at distinct sites, according to new research published in *Nature Communications*. The findings of this study shed light on the opposing, non-redundant roles of these two kinases in regulating homeostasis and inflammation.

The fine-tuning of signalling pathways downstream of immunoreceptor tyrosine-based activation motif (ITAM)-bearing receptors, such as T cell receptors, B cell receptors and Fc receptors, is important in maintaining homeostasis within the immune system. The binding of high-avidity ligands to these receptors induces activating signals, whereas the binding of low-avidity ligands can induce inhibitory signals by a mechanism known as inhibitory ITAM (ITAMi) signalling, in which SHP1 activity has been implicated.

> “FYN and LYN differentially control SHP1 activity by regulating its phosphorylation status”



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To investigate the switch between ITAMi and ITAM signalling, Ben Mkaddem and colleagues focused on LYN and FYN. In both monocytic and lymphocytic cell lines cultured under ITAM and ITAMi-inducing conditions, *in vitro* knockdown of LYN expression inhibited ITAMi signalling but had no effect on ITAM signalling. Conversely, silencing FYN expression had no effect on ITAMi signalling, but converted ITAM signalling into ITAMi signalling.

The inhibitory signalling generated by silencing FYN was abolished when both FYN and SHP1 were knocked down, indicating a link between FYN and SHP1. In bone marrow-derived macrophages, the researchers found that FYN directed the phosphorylation of SHP1 at S591, whereas LYN directed the phosphorylation of SHP1 at Y536. Phosphorylation of SHP1 at S591 and Y536 is associated with the inhibition and activation of SHP1, respectively, suggesting that FYN and LYN differentially control SHP1 activity by regulating its phosphorylation status.

To explore the functional role of this regulation *in vivo*, Ben Mkaddem and colleagues used two mouse models: a model of nephrotoxic serum nephritis and the collagen antibody-induced arthritis (CAIA) model. LYN deficiency exacerbated nephritis and arthritis, and was associated with phosphorylation of SHP1 at S591, whereas FYN deficiency was protective and associated with SHP1 phosphorylation at Y536. In the CAIA model, treating transgenic mice, expressing either human Fcγ receptor IIA (hFcγRIIA) or human Fcα receptor I (hFcαRI), with antibodies that induce ITAMi signalling prevented disease development; this protection required LYN expression.

Translating these findings into humans, the researchers found that LYN, but not FYN, was recruited to FcγRIIA in the leukocytes of healthy individuals, which was associated with phosphorylation of SHP1 at Y536. However, the leukocytes of patients with lupus nephritis only weakly recruited LYN, but strongly recruited FYN to FcγRIIA, a situation that was associated with phosphorylation of SHP1 at S591. These findings support the role of LYN and FYN in controlling the balance between homeostasis and inflammation; an imbalance that could lead to the development of autoimmune diseases.

Jessica McHugh

ORIGINAL ARTICLE Ben Mkaddem, S. *et al.* Lyn and Fyn function as molecular switches that control immunoreceptors to direct homeostasis or inflammation. *Nat. Commun.* http://dx.doi.org/10.1038/s41467-017-00294-0 (2017)

Nature Reviews Rheumatology | Published online 31 Aug 2017; doi:10.1038/nrrheum.2017.145

RHEUMATOID ARTHRITIS

Characterization of the infrapatellar fat pad

> IFP tissue isolated from patients with RA contained more inflammatory cells than IFP tissue from patients with OA

The infrapatellar fat pad (IFP), a fat depot in the knee joint, is thought to have a role in the pathogenesis of osteoarthritis (OA). A new study now characterizes the IFP in rheumatoid arthritis (RA) for the first time.

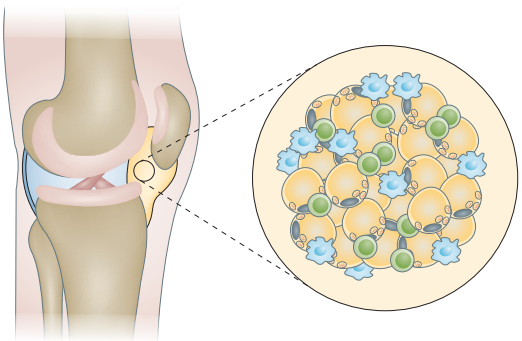
Studies in OA have shown that intra-articular adipose tissue has a distinct, more pro-inflammatory phenotype than adipose tissue elsewhere in the body, and is suggested to contribute to OA pathogenesis by promoting synovial inflammation and modulating cartilage degradation. "Because most RA joint tissues display a more inflammatory phenotype than their OA counterparts," explains Andreea Ioan-Facsinay, the corresponding author, "we expected to find the same for the IFP."

To this end, the authors compared IFP and synovial tissue of patients with RA ($n = 20$) and OA ($n = 51$). Both the inflammatory cells infiltrating the IFP and synovium and the adipokines and cytokines secreted by these tissues were determined.

As expected, IFP tissue isolated from patients with RA contained more inflammatory cells than IFP tissue from patients with OA, but the levels and types of adipocytokines secreted were not significantly different. These findings suggest that the infiltrating immune cells contribute little to the secretion of adipocytokines by the IFP. Although the number of mast cells was increased in IFP tissue of patients with RA compared with OA, the opposite finding was observed in synovial tissue. "This observation indicates a differential regulation and possible role of mast cells in the synovium," states Ioan-Facsinay.

Ioan-Facsinay highlights that this study is the first one to report on IFP tissue in RA and suggests that the IFP has potentially a different role in RA compared with OA. However, validation with more samples and in different disease stages is required.

Liesbet Lieben, Senior Editor,
Nature Reviews Disease Primers



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ORIGINAL ARTICLE(S) de Jong, A. J. *et al.* Inflammatory features of infrapatellar fat pad in rheumatoid arthritis versus osteoarthritis reveal mostly qualitative differences. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211673 (2017)

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Nature Reviews Rheumatology | Published online 24 Aug 2017; doi:10.1038/nrrheum.2017.144

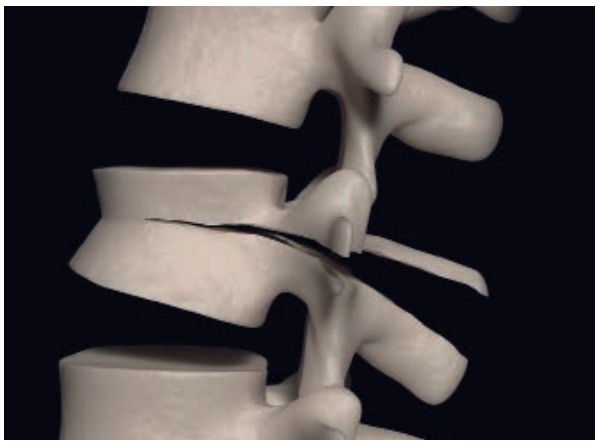
OSTEOPOROSIS

Discontinuing denosumab discouraged

> …there might be an increased risk of multiple vertebral fractures in patients who had discontinued denosumab

The discontinuation of denosumab, a biologic therapy that targets receptor activator of NF-κB ligand (RANKL; also known as TNFSF11), in the treatment of patients with osteoporosis has been discouraged in a new position paper from the European Calcified Tissue Society (ECTS).

"We noticed that there is a great need for advice on the duration of treatment with denosumab," states Carola Zillikens, corresponding author on the position paper. "Some recent case reports and series suggest that denosumab discontinuation may lead to an increased risk of multiple vertebral fractures," she explains. "In order to provide advice on management, ECTS formed a working group and reviewed existing literature on the effects of stopping denosumab."



David Marchal/Alamy Stock Photo

Denosumab inhibits osteoclast function, thereby reducing bone resorption, but unlike some osteoporosis therapies, denosumab does not remain in the bone for long periods of time. The advice for most patients taking oral skeletally retained therapies, such as oral bisphosphonates, is to discontinue or have a break from taking the therapy after 5 years to avoid serious adverse effects. However, it seems that in clinical practice, these guidelines are also being applied to patients taking denosumab.

"Denosumab withdrawal quickly reverses the positive effects of the drug concerning bone mineral density, bone turnover markers and bone microarchitecture structure, and these changes may possibly be associated with an increased risk of multiple vertebral fractures," explains Elena Tsourdi, first author on the position paper. "This is a different situation from bisphosphonates, which have a persistent effect after discontinuation due to their high affinity for binding hydroxyapatite."

The ECTS working group evaluated data from 25 relevant publications, abstracts and clinical trials. In particular, data from the FREEDOM and FREEDOM Extension Trial indicated that there might be an increased risk of multiple vertebral fractures in patients who had discontinued denosumab compared with the placebo group.

"Based on available evidence, we advise that a re-evaluation should be performed after 5 years of denosumab treatment," says Zillikens. "Patients considered at high fracture risk should either continue denosumab therapy for up to 10 years or be switched to an alternative treatment. For patients at low risk, denosumab could be discontinued after 5 years but bisphosphonate therapy should be considered to reduce or prevent the rebound increase in bone turnover."

Joanna Collison

ORIGINAL ARTICLE Tsourdi, E. *et al.* Discontinuation of denosumab therapy for osteoporosis: a systematic review and position statement by ECTS. *Bone* http://dx.doi.org/10.1016/j.bone.2017.08.003 (2017)

Nature Reviews Rheumatology | Published online 7 Sep 2017; doi:10.1038/nrrheum.2017.151

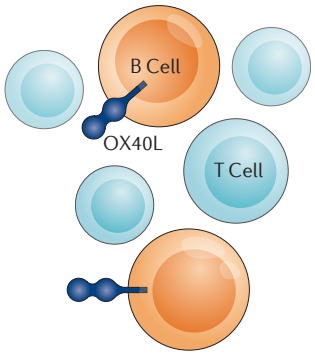
SYSTEMIC LUPUS ERYTHEMATOSUS

OX40L-expressing B cells promote SLE

> OX40L on B cells supports plasma cell development and [T follicular helper] cell maturation

OX40 ligand (OX40L) supports the development of T follicular helper (T_{FH}) cells via its expression on B cells, according to new research. "We studied OX40L because the gene *TNFSF4* (that encodes OX40L) is a risk gene for the development of systemic lupus erythematosus (SLE)," explains corresponding author Timothy Vyse. The results of this study shed light on the mechanisms by which high levels of OX40L predispose individuals to SLE.

To investigate the function of OX40L on T cells and B cells, the researchers developed three knockout mouse strains: OX40L germline knockout mice and mice lacking OX40L in either their $CD4^+$ T cells or in their B cells. Following immunization with a T cell-dependent antigen, all three knockout strains had reduced primary humoral responses compared with control mice. However, mice with OX40L-deficient B cells also had impaired secondary humoral responses, unlike mice with OX40L-deficient $CD4^+$ T cells, suggesting a distinct role for OX40L on B cells.



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As OX40L has a well-established role in T cell activation, the researchers analysed the splenic T cell composition of these three mouse strains. As expected, immunized OX40L-deficient mice had lower proportions of T effector cells than control mice; this defect was evident in all three knockout strains and could not explain the difference in secondary responses, indicating a role for B cell OX40L that is independent of T cell activation.

Following immunization, mice with OX40L-deficient B cells had lower percentages of plasma cells and fewer germinal centre T_{FH} cells than control mice. By contrast, no differences were observed in mice with OX40L-deficient $CD4^+$ T cells, indicating that OX40L on B cells supports plasma cell development and T_{FH} cell maturation.

In two mouse models of SLE — a congenic model and a graft-versus-host model — OX40L deficiency ameliorated disease and was accompanied by a reduction in anti-dsDNA autoantibodies, kidney deposition of immunoglobulin and germinal centre T_{FH} cell and plasma cell numbers. Similar findings were observed in the graft-versus-host model in mice with OX40L-deficient B cells, suggesting that OX40L predisposes to SLE via its expression on B cells. "These findings describe one mechanism by which excess OX40L activity promotes disease, namely B cell OX40L promotes T_{FH} cell generation," concludes Vyse.

Jessica McHugh

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Nature Reviews Rheumatology | Published online 31 Aug 2017; doi:10.1038/nrrheum.2017.148

RHEUMATOID ARTHRITIS

Cell cycle stalling linked to arthritis

> In the K/B×N serum transfer model of murine arthritis, mice deficient for LBH had more severe disease…

A single nucleotide polymorphism (SNP) in the enhancer region of *LBH* (which encodes the transcriptional cofactor protein LBH) causes low levels of LBH expression and is associated with rheumatoid arthritis (RA), although the mechanisms involved in this association are unknown. Now, a new study has revealed a role for LBH in progression through the cell cycle and in preventing the accrual of DNA damage.

"Our decision to focus on LBH comes from our unbiased informatics approach," explains corresponding author Gary S. Firestein. "LBH not only appeared in multiple datasets, but also has SNPs associated with other immune-mediated diseases," he continues. "We felt that the computational methods were telling us something important about autoimmunity and how *LBH* might be a seminal gene at the centre of immune dysregulation."

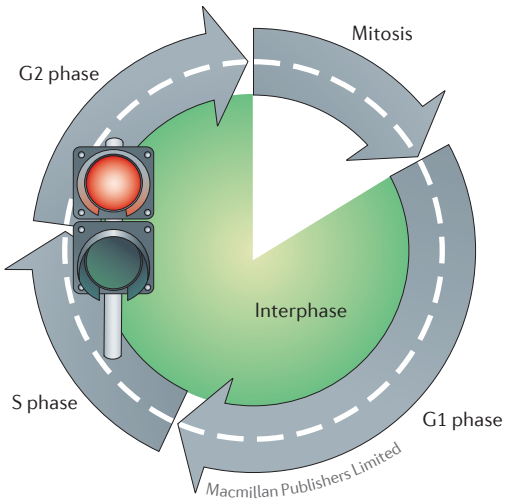
The research team explored the role of LBH in the cell cycle of fibroblast-like synoviocytes (FLS) from patients with RA. FLS with small interfering RNA (siRNA)-induced LBH deficiency failed to progress through the cell cycle, remaining in S phase for ≥72h. Delayed cell cycle progression in LBH-deficient FLS was associated with increased levels of DNA damage compared with scrambled siRNA-transfected control FLS. In addition, checkpoint kinase CHK1 was hyperphosphorylated and the expression of DNA polymerase α was decreased in LBH-deficient FLS compared with controls, suggesting that the accumulation of DNA damage in these cells could lead to activation of the S phase checkpoint and cell cycle arrest.

"A defect in cell division as a mechanism of autoimmunity seems counter-intuitive because immune diseases are usually thought to result from increased proliferation," states Firestein. "However, defects in DNA polymerase due to LBH deficiency led to the accumulation of DNA fragments, which in other systems is known to cause arthritis."

In the K/B×N serum transfer model of murine arthritis, mice deficient for LBH had more severe disease and increased levels of IL-1β compared with wild type mice. These results suggested to the authors that defective proliferation rather than just increased proliferation is important in the pathogenesis of RA.

"This is one of the few genes in RA where a disease-associated SNP is functional, and the results could provide clues about why LBH polymorphisms are associated with so many autoimmune diseases," remarks Firestein.

Joanna Collison



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RHEUMATOID ARTHRITIS

Remission — keeping the patient experience front and centre

Lilian H. D. van Tuyl and Maarten Boers

Interpreting existing patient-reported outcome measures for the experience of remission by patients with rheumatoid arthritis is not straightforward. The challenge is to find a better, more accurate measure.

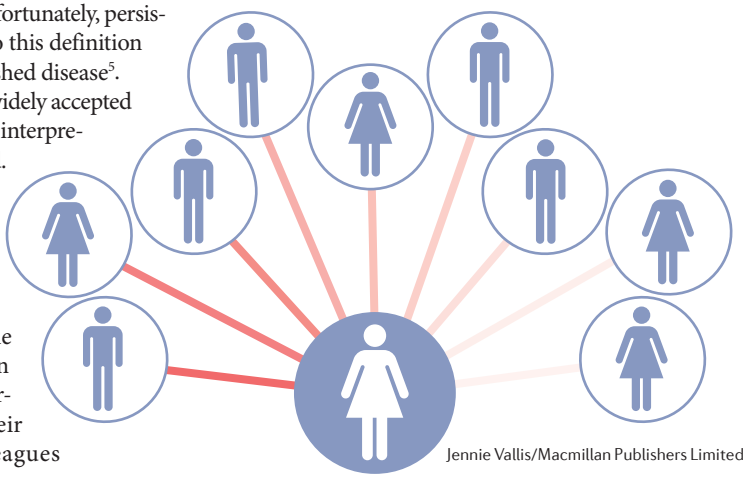
Refers to Ferreira, R. J. O. *et al.* Suppressing inflammation in rheumatoid arthritis: does patient global assessment blur the target? A practice-based call for a paradigm change. *Arthritis Care Res. (Hoboken)* http://dx.doi.org/10.1002/acr.23284 (2017)

In clinical epidemiology and outcomes research, measuring disease activity and severity are of utmost importance for classification and evaluation purposes. Such research facilitates clinical decision making and improves the quality of health care. Patient-reported outcomes (PROs) are typically used to categorize patients according to their disease activity or severity, with the patient being the primary source of information on the effect of the disease and the PRO tool being viewed as the ultimate outcome. However, PROs are often criticized for being subjective, variable, influenced by external factors and, therefore, easily biased. One PRO in particular — the patient global assessment (PGA) — has been the subject of some debate, and new research from Ferreira *et al.*[1] shines more light on the issues around using PROs to guide treatment.

In 2011, the ACR and EULAR proposed new criteria for assessing remission in patients with rheumatoid arthritis (RA), comprising four criteria that included the PGA as the only PRO[2]. The PGA is a tool to assess patient-perceived disease activity whereby the patient responds to a question about how they are affected on a scale of either 0–10 or 0–100, with higher scores representing worse disease state. The PGA is able to discriminate active treatment from control treatment equally as well as, and sometimes better than, more objective measures of disease activity, such as levels of C-reactive protein or swollen joint count[2]. Nevertheless, criticism abounds regarding the inclusion of the PGA in the remission criteria for RA and regarding the appropriate threshold for remission; the main arguments are that the PGA does not (fully) reflect the inflammatory process because it can be influenced by comorbidities, such as osteoarthritis and pain syndromes, and that the threshold (≤1 on a scale of 0–10) is too low because respondents rarely use the extremity of the scale even if they feel extremely well[3]. Even though the remission criteria were primarily intended for classifying patients in clinical research (not for use in the clinic), the committee charged with making the criteria decided to develop a specific definition, even at the expense of sensitivity. Later research has demonstrated that this definition is indeed highly specific, with several studies subsequently showing that patients with RA in remission are 'normal': such patients function normally in work and social roles and their disease does not progress[4]. Unfortunately, persistent remission according to this definition is rare, especially in established disease[5].

Although the PGA is a widely accepted measure in RA research, its interpretation is not straightforward. Now, Ferreira *et al.*[1] have built on earlier efforts by the Outcome Measures in Rheumatology (OMERACT) working group, which identified the patient's perspective on remission in RA as an important research area[6]. In their work, Ferreira and colleagues studied the clinical and psychological determinants of the PGA score within the ACR–EULAR Boolean-based remission definition[1]. In line with previous studies, Ferreira *et al.* showed that a PGA score ≤1 was the most difficult of the criteria in the remission definition to fulfil. In addition, they demonstrated that in patients with a PGA score >1 who are otherwise in remission, half of the PGA score reflects fatigue, pain, anxiety and loss of function, whereas the remaining variability in the PGA score remains unexplained[1]. As many of these symptoms can be improved with effective antirheumatic treatment, whether their cause is due to comorbidities or ongoing inflammation remains unclear. After all, imaging techniques such as ultrasonography and MRI are frequently able to detect subclinical inflammation in patients with RA who are in remission[7]. Regardless of whether the PGA score reflects residual disease activity or not, patients with a score >1 require treatment for fatigue, pain, anxiety and function to achieve ACR/EULAR remission.

With this new information in hand, there are at least two ways to proceed. First, we might decide that including the patient perspective in disease management is too complicated and remove the PGA from the remission definition. Accordingly, rheumatologists would focus on tight control of inflamed joints and acute phase reactants alone. Or, we could make an effort to understand what patients experience in remission, to measure this experience more appropriately and accurately than with the PGA (alone) and to capture this information in the definition of remission.



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Our preference is to understand remission better: a feasible but challenging target aimed at consensus between patients and physicians, underpinned by the conviction that a patient should be seen and treated as a whole. Research is already well underway; in collaboration with EULAR, we have conducted qualitative research to understand which domains of health substantially contribute to the patient's experience of low disease activity[8]. On the basis of this qualitative research, patients with RA from the Netherlands, United Kingdom, United States, Austria, Denmark and France prioritized three domains as essential in a state of remission: pain, fatigue and independence[9]. Currently, an international validation study to identify appropriate measurement instruments for these domains in low disease activity is ongoing and will provide information not only on the feasibility of measuring the domains of pain, fatigue and independence in low disease activity, but also on the added value of these domains in correctly classifying patients as being in remission or not.

Although Ferreira *et al.*[1] suggest removing the PGA from the ACR/EULAR remission definition, the Patient Perspective on Remission in RA special interest group that gathered at the 13th OMERACT conference in 2016 expressed a preference for modifying the criteria. In particular, this group favoured adding patient-reported domains, or switching the PGA for one or more patient-important domains[10].

We agree with Ferreira and colleagues that the PGA is not the optimal way to capture the complete picture of disease remission[1]. However, calling for a paradigm change that limits the responsibility of the rheumatologist to prescribing immunosuppressive therapy and measuring three clinical variables seems neither challenging nor effective. We are convinced that most rheumatologists in practice do not need new instruments to decide which patients are most likely have residual disease and are in need of switching their treatment as opposed to patients with comorbidities that confound the interpretation of their RA symptoms. As Ferreira *et al.* mention, "controlling the impact of disease upon patients' lives is the core objective of disease management"[1]. Taking away the incentive to improve RA care by removing the patient's perspective from the remission criteria does not contribute to reaching this objective. Instead, let us be ambitious, accept that we can't yet make all patients feel well and focus our energy towards improving the care of patients in the direction of the ultimate goal — remission.

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SPONDYLOARTHROPATHIES

Ruminococcus on the horizon in arthritic disease

Lars Vereecke and Dirk Elewaut

Increasing evidence points to a mechanistic link between gut and joint pathology as the gut contains the largest number of immune cells of any tissue and trillions of commensals that contribute to immune development and homeostasis. New research is putting the role of *Ruminococcus gnavus* in arthritic disease in the spotlight.

Refers to Breban, M. *et al.* Faecal microbiota study reveals specific dysbiosis in spondyloarthritis. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-211064 (2017)

Spondyloarthritis (SpA), a multifactorial disease influenced by genetic, immunological and environmental factors, can affect both the axial and peripheral skeleton and is often associated with extra-articular manifestations such as uveitis, psoriasis and gut inflammation. Remarkably, 50% of patients with SpA can have intestinal inflammation without overt gastrointestinal symptoms (known as subclinical inflammation). Such microscopic gut inflammation is a risk factor for active SpA and is associated with the risk of structural disease progression, the degree of spinal inflammation and the risk of developing Crohn's disease[1]. The progression and severity of SpA are strongly affected by environmental factors, including the intestinal microbiome[2]; however, the mechanisms underlying the link between gut and joint pathologies are not completely understood.

In a new study, Breban *et al.*[3] set out to characterize disease-specific microbiota profiles by sequencing 16S ribosomal RNA from stool samples of patients with SpA ($n = 96$), patients with rheumatoid arthritis (RA; $n = 32$) and healthy individuals ($n = 71$, including 43 siblings of patients with SpA). The researchers observed disease-specific intestinal dysbiosis and reduced microbial diversity in patients with SpA and patients with RA compared with healthy individuals. Additionally, patients with SpA had an increased prevalence of *Ruminococcus gnavus* in their intestines compared with patients with RA and healthy individuals, which positively correlated with disease activity in those patients who had a history of inflammatory bowel disease (IBD)[3]. Breban *et al.* also identified differences in the composition of the intestinal microbiota between healthy siblings of patients with SpA who were HLA-B27-positive and those who were HLA-B27-negative, indicating that genetic factors influence gut microbiota composition in both health and disease. Overall, this study[3] strengthens the idea that human arthritides are associated with specific changes in the intestinal microbiota (FIG. 1).

Growing evidence suggests that autoimmunity might originate in the intestine. Over 1,000 intestinal bacterial species have been identified to date, and each individual gut is thought to house approximately 160 different species. The intestinal microbiome is estimated to contain around 3 million genes, reflecting the enormous metabolic and translational capacity of the gut microbiota. Bacteria-derived metabolites, including short-chain fatty acids and tryptophan metabolites, affect the integrity of the gut epithelial barrier and are linked to arthritis[4]. In addition, microbial-derived peptides can share homology with host-derived self-peptides, which can potentiate the development of autoimmunity — a phenomenon known as molecular mimicry. For example, a study published in 2017 showed that *N*-acetylglucosamine-6-sulfatase (GNS) and filamin A are highly expressed in the synovium of patients with RA[5]. Peptides derived from GNS and presented by HLA-DR molecules had marked sequence similarity to epitopes of sulfatase proteins found in *Prevotella* spp. and *Parabacteroides* spp., whereas HLA-DR-presented peptides derived from filamin A shared high sequence similarity with epitopes of sulfatase proteins from *Prevotella* spp. and *Butyricimonas* spp.[5]. This molecular mimicry provides an additional link between the intestinal microbiota and autoimmune joint pathology. In two separate cohorts of patients with RA, Breban *et al.* found reduced bacterial diversity and dysbiosis[3].

Breban *et al.* described how the intestinal microbial profiles of patients with SpA differ from those of patients with RA and healthy individuals, with each group showing specific changes in the prevalence of bacterial groups at the family, genus or species level[3]. The inherent limitation of such descriptive association studies is the lack of evidence regarding a direct causal relationship between microbiota and disease. In cases in which distinct microbial profiles seem to contribute to disease, it is assumed that overrepresented species have pathogenic characteristics, whereas underrepresented species confer protection. For example, in this study[3], the mucolytic bacteria *R. gnavus* was significantly overrepresented in the guts of patients with SpA who had active disease, defined by a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score ≥3 ($P = 0.034$). Interestingly, although the prevalence of *R. gnavus* was even greater in those patients with active SpA who had a history of IBD, the prevalence of these bacteria was not related to IBD disease activity. *R. gnavus* was also mor prevalent in patients with SpA compared with their healthy siblings, who share a similar genetic background and usually share a similar history of environmental exposure. A pioneering study published earlier this year also showed intestinal dysbiosis in mucosal biopsy samples from patients with SpA; subclinical gut inflammation and expansion of the bacterial genus *Dialister* positively correlated with disease activity as measured by the Ankylosing Spondylitis Disease Activity Score (ASDAS) in these patients[6].

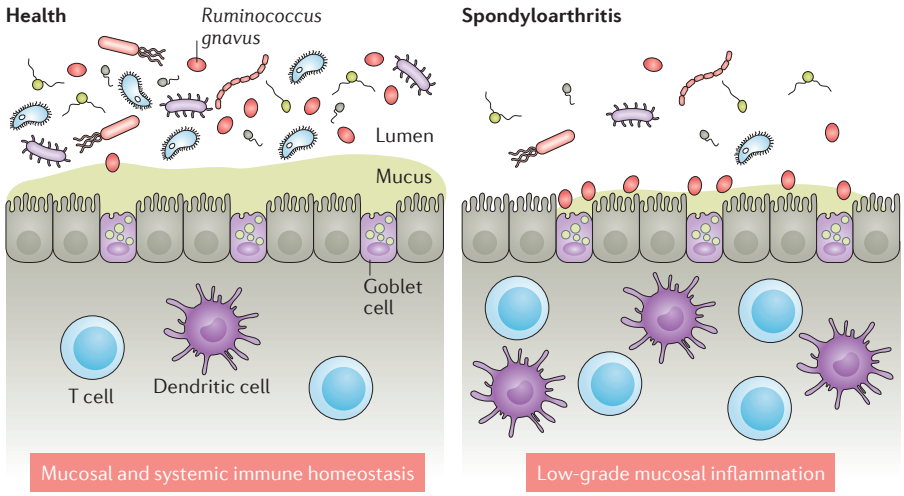

Figure 1 | **Disturbed microbiota–host interactions in spondyloarthritis.** The intestines of healthy individuals have high levels of bacterial diversity with a very low prevalence of *Ruminococcus gnavus*, contributing to a stable intestinal barrier and to homeostasis of the mucosal and systemic immune system. By contrast, the intestines of patients with spondyloarthritis have reduced bacterial diversity and high abundance of *R. gnavus*. This high prevalence of *R. gnavus* is thought to lead to mucus degradation, destabilization of the intestinal barrier, low-grade mucosal inflammation and spondyloarthritis.

R. gnavus is a strict anaerobic Gram-positive bacterium of the Lachnospiraceae family that degrades mucus by expressing intramolecular trans-sialidases, thereby providing itself with a unique carbon source[7]. Mucus production by intestinal goblet cells contributes to the integrity of the intestinal epithelial barrier by forming an impermeable gel layer containing high concentrations of antimicrobial peptides and IgA; however, the outer mucus layer is permeable to the microbiota and represents an ecological niche for bacteria, such as *R. gnavus*, which can attach to mucins or use these glycoproteins as a nutritional source. Interestingly, the prevalence of another mucolytic species, *Akkermansia muciniphilia,* is decreased in individuals with obesity or type 2 diabetes mellitus: conditions associated with low-grade intestinal inflammation[8]. The presence of *A. muciniphilia* is also associated with the development of arthritis in HLA-B27 transgenic rats and in children with enthesitis-related arthritis[9]. Specific types of mucolytic bacteria could be particularly harmful if they are able to contribute to intestinal barrier impairment and inflammatory joint disease. In this regard, *R. gnavus* expresses ß-glucuronidase, an enzyme that can generate toxic metabolites[10] and can metabolize bile acids into pro-inflammatory secondary bile acids, including deoxycholic and lithocholic acids, which are associated with intestinal toxicity[3].

Breban *et al.* strategically focused on stool samples, which enabled them to obtain a large volume of samples through non-invasive methods. Additional analysis of intestinal biopsies to quantify *R. gnavus* abundance and histological examination of carnoy-fixed samples to study bacterial–epithelial interactions in mucus-preserving conditions would be very informative. Pathological analysis of such intestinal biopsies could reveal whether subclinical gut inflammation is correlated with *R. gnavus* abundance. Mechanistic studies assessing whether the presence of *R. gnavus* is linked with loss of epithelial barrier integrity are also needed to establish a direct causal relationship. Together, these findings provide substantial new insights into the diverse mechanisms by which the intestinal microbiota contribute to arthritic diseases.

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OSTEOARTHRITIS

Chondroitin sulfate — CONCEPT clear, uncertainties unchanged

Gabriel Herrero-Beaumont and Raquel Largo

The newly published findings from the Chondroitin Versus Celecoxib Versus Placebo Trial (CONCEPT) underscore the complexity of performing clinical trials in the field of knee osteoarthritis. But do the results of CONCEPT merit the consideration of chondroitin sulfate as a first-line therapy?

Refers to Reginster, J., Dudler, J., Blicharski, T. & Pavelka, K. Pharmaceutical-grade chondroitin sulfate is as effective as celecoxib and superior to placebo in symptomatic knee osteoarthritis: the ChONdroitin versus CElecoxib versus Placebo Trial (CONCEPT). *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210860 (2017)

Whether symptomatic slow-acting drugs for osteoarthritis (SYSADOA), such as chondroitin sulfate, should be recommended for the management of knee osteoarthritis (OA) is an area of great contention. In the Chondroitin Versus Celecoxib Versus Placebo Trial (CONCEPT), treatment with either chondroitin sulfate or celecoxib (a cyclooxygenase-2 selective inhibitor) was superior to placebo in reducing pain and improving function in patients with symptomatic OA after 6 months[1]. Should chondroitin sulfate be considered a first-line pharmacological option, as the investigators of CONCEPT suggest?

CONCEPT is the first clinical trial to be conducted in full accordance with the 2015 European Medicines Agency (EMA) guidelines on clinical investigation in OA[2]. In this sense, CONCEPT could be considered a proof-of-concept clinical trial. The approach taken by Reginster *et al.*[1] is of considerable clinical interest as it enables researchers to not only study the efficacy of a drug, but also to test the accuracy of the new guidelines.

As well as the EMA guidelines, additional efforts have been made by other investigators to ensure patient safety and data accuracy in OA clinical trials. For example, the Data and Safety Monitoring Board (DSMB) is an independent committee that assures the methodological stringency of a trial by outlining the sample-adjusted statistical analysis to be used and the type of comparison to be conducted, as well as providing researchers with a well-defined primary endpoint with a minimum clinically significant difference and rescue medication to consider using[3]. These different aspects, however, were not completely discussed in the study by Reginster and colleagues.

Although a substantial placebo effect has been previously reported in OA trials, the size of the placebo effect in CONCEPT was notable[1]. The 2015 EMA guidelines contributed critically in this study[1] by highlighting the need to initially assess the placebo effect size to indicate what sample size of the population would be needed for clinically significant results, and hence, put the yielded results into context. However, in OA research, we need to be aware that a statistically significant result is not necessarily a strong and robust result. Statistical significance conveys little information when measurements are taken in noisy conditions, and might even overestimate the size of an effect[4].

In CONCEPT, the efficacy shown for chondroitin sulfate after 6 months of treatment was intriguingly similar to that observed with celecoxib[1]. Moreover, the same pattern of pain improvement was observed in the three intervention arms, including placebo, after 1 and 3 months of treatment. This finding differs from the expected time curve response of NSAIDs. According to former knee OA clinical trials exploring pain relief using a variety of NSAIDs, the effect of NSAID therapy should be evident within a few weeks of treatment commencing, and at an earlier time point than 3 months[5]. Thus, the fact that celecoxib did not show an analgesic effect during the first 3 months of treatment, but demonstrated efficacy after 6 months in CONCEPT, is puzzling.

Similarly, a meta-analysis conducted by the Cochrane Collaboration concluded that treatment with chondroitin sulfate improves clinical symptoms in patients with OA in the short-term (<6 months) compared with placebo or a comparator oral medication, with an absolute risk difference of 10%[6]. In fact, the randomized trials included in this analysis revealed that chondroitin sulfate was better than placebo in studies of 3 months duration or less. As a SYSADOA, chondroitin sulfate would be expected to have an accumulative analgesic effect over time, and not to only show efficacy at the last visit (that is, at 6 months).

These reflections led us to look more closely at the study population. Although equally distributed among the three groups, 75% of the patients in CONCEPT had mild disease, as defined by a Kellgren-Lawrence grade of 1 or 2 (REF. 1). Such a population is likely to be more heterogeneous than one containing patients with more severe disease and higher pain levels (indicative of later stages of disease) (FIG. 1), which could result in a more varied drug response. Notably, to be included in the study, patients only needed to have experienced pain for >3 months before enrolment, which might potentially favour the inclusion of patients in the early phases of OA. These potential caveats raise the question of what kind of OA population should be included in a clinical trial simultaneously comparing the analgesic effect of a SYSADOA and a cyclooxygenase-2 selective inhibitor, as each of these drugs is likely to be effective in particular subgroups of patients[7].

Whether the substantial reduction of pain observed in the three groups at 1 and 3 months in this study could be explained by spontaneous improvements of incidental bone marrow oedema, tendon strains or other lesions associated with knee OA should be taken into consideration. The placebo effect and the apparent efficacy of the drugs could be partially explained by factors inherent to the natural course of the disease in the selected population (FIG. 1). In light of these

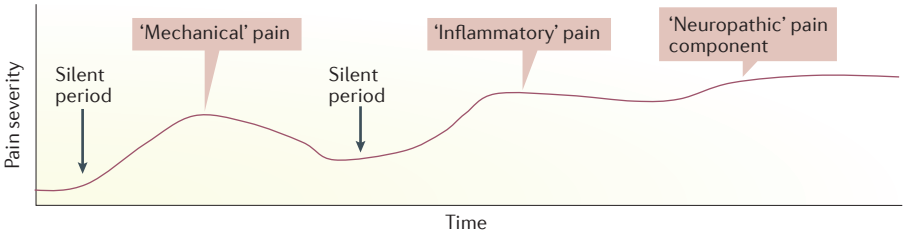

Figure 1 | **Variations in osteoarthritis pain characteristics and severity over time.** The complexity of osteoarthritis (OA) hampers the evaluation of treatment responses. OA is heterogeneous, presenting with different phenotypes in each patient according to the predominant joint tissue involved at a particular time. However, in the advanced stages of the disease, OA evolves towards a more uniform disease with persistent pain, sometimes with neuropathic characteristics. The transition from early to advanced disease might include silent periods, which could explain high rates of improvement in some clinical trials.

data, we wonder whether the subpopulation that remained symptomatic at the end of CONCEPT differed from the baseline cohort?

In CONCEPT, use of chondroitin sulfate did not result in a statistically significant number of adverse events or withdrawals due to adverse events compared with the other treatment arms. However, the number of patients in the chondroitin sulfate group who discontinued the trial seemed to be higher (39 patients out of 199 (19.6%) in the chondroitin sulfate group versus 27 patients out of 200 (13.5%) in the celecoxib group), which could have added statistical noise[4]. So far, a limited range of adverse events has been ascribed to chondroitin sulfate use, with only some studies reporting their occurrence[6]; however, in daily practice, patients frequently complain of abdominal symptoms, a finding that has occasionally been reported in clinical trials[3].

When investigating the therapeutic potential of chondroitin sulfate, the preparation itself must also be considered. Chondroitin sulfate can be provided either as a pharmaceutical-grade or as a nutraceutical-grade formulation. The latter likely exhibits large variations between preparations[1], but as an organic molecule, even the pharmaceutical-grade chondroitin sulfate is subject to manufacture-related modifications. Each batch has its own chondroitin sulfate molecular weight and 4-to-6 isomer ratio[8]. This fact hampers the performance of chondroitin sulfate in *in vitro* studies and might also have consequences in clinical research. Even so, the anti-inflammatory effect of intraperitoneally administered pharmaceutical-grade chondroitin sulfate has been demonstrated in a rabbit experimental model of chronic arthritis[8,9]. Unfortunately, studying the pharmacokinetics of the compound is not possible because chondroitin sulfate is not detectable in the serum after oral administration.

In conclusion, CONCEPT is undeniably an excellent study that once again underscores the complexity of performing clinical trials in the field of knee OA; however, methodological uncertainties remain. Thus, we do not believe the results presented by CONCEPT support the consideration of chondroitin sulfate as a first-line pharmacological option for patients with knee OA.

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The authors declare no competing interests.

Giant cell arteritis and polymyalgia rheumatica: current challenges and opportunities

Christian Dejaco[1,2], Elisabeth Brouwer[3], Justin C. Mason[4], Frank Buttgereit[5], Eric L. Matteson[6] and Bhaskar Dasgupta[7]

Abstract | The fields of giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) have advanced rapidly, resulting in a new understanding of these diseases. Fast-track strategies and improved awareness programmes that prevent irreversible sight loss through early diagnosis and treatment are a notable advance. Ultrasonography and other imaging techniques have been introduced into routine clinical practice and there have been promising reports on the efficacy of biologic agents, particularly IL-6 antagonists such as tocilizumab, in treating these conditions. Along with these developments, which should improve outcomes in patients with GCA and PMR, new questions and unmet needs have emerged; future research should address which pathogenetic mechanisms contribute to the different phases and clinical phenotypes of GCA, what role imaging has in the early diagnosis and monitoring of GCA and PMR, and in which patients and phases of these diseases novel biologic drugs should be used. This article discusses the implications of recent developments in our understanding of GCA and PMR, as well as the unmet needs concerning epidemiology, pathogenesis, imaging and treatment of these diseases.

Giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) are overlapping inflammatory rheumatic disorders commonly affecting older people[1]. Although reports by Horton (in 1932)[2], Paulley and Hughes (in 1960)[3] and Hamrin (in 1972)[4] already recognized the systemic nature of GCA, clinicians have generally viewed GCA as a predominantly 'headache disease' characterized by cranial symptoms. This perception was perhaps fostered by the 1990 ACR classification criteria for GCA, which have frequently been misused for diagnosing this disease. The ACR criteria focus on cranial symptoms such as headache and swelling and/or tenderness of the temporal artery[5,6]. However, routine use of vascular imaging has demonstrated that large-size vessels are involved in GCA more frequently than previously thought, leading to a broader understanding of GCA as a 'vasculitic' syndrome that includes large-vessel vasculitis and PMR[6].

Large-vessel GCA (LV-GCA), a subset of GCA, affects large, supra-aortic arteries, their branches and/or the aorta and it is frequently discovered on vascular imaging studies conducted in patients with difficult-to-treat polymyalgia and/or with constitutional symptoms such as weight loss, night sweats and fever of unknown origin[1,6]. LV-GCA-related arterial stenosis can result in upper limb claudication. Aortic inflammation is often associated with constitutional symptoms and can lead to the formation of aneurysms that cause abdominal, thoracic and/or back pain if complicated by intramural haematoma, dissection or rupture[7]. PMR is clinically characterized by aching and stiffness in the cervical region, shoulder and pelvic girdles[8].

The most feared complication of GCA is irreversible sight loss. Cerebrovascular strokes, infarction of the tongue and scalp necrosis are less common complications of this disease[9]. Permanent visual loss caused by anterior ischaemic optic neuropathy (AION) occurs in 15–20% of patients with GCA[10,11]; improved diagnosis and prompt initiation of therapy has reduced the ischaemic complications of GCA, including those associated with sight[12,13].

Glucocorticoids are the standard treatment for GCA and PMR even though glucocorticoid-related adverse events occur in up to 85% of treated cases[14]. Many patients have pre-existing co-morbidities that may pose relative or absolute contraindications to glucocorticoid therapy. The prevalence of flares is high, and it is related to the dose and duration of glucocorticoid therapy. In cohort studies flares were observed in 34–62% of patients[15–17], and the results of clinical trials in which glucocorticoid treatment was rapidly tapered

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Key points

- Giant cell arteritis (GCA) is best understood as an inflammatory vascular syndrome with features of cranial and/or large-vessel vasculitis, systemic inflammation and polymyalgia rheumatica (PMR), which frequently overlap
- GCA and PMR are among the most common inflammatory rheumatic diseases in the elderly; the prevalence of these diseases is expected to increase due to ageing of the population
- The role and value of imaging in GCA and PMR is evolving quickly
- The pathophysiology of GCA is characterized by phases of initiation, transmural inflammation and chronic vessel wall injury and repair, each of which might be novel drug targets
- Glucocorticoids are the standard-of-care treatment for GCA and PMR, although methotrexate is used in individual cases and anti-IL-6 therapy is now approved for the treatment of GCA
- The selection of patients for biologic DMARD therapy, defining the best treatment strategies and the development of reliable outcome parameters are challenges in the future management of GCA and PMR

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suggest that sustained remission is achieved in only 15–20% of patients treated with glucocorticoids alone[18,19]. Methotrexate, in combination with glucocorticoids, can be used to treat individuals with GCA and PMR[20–23]; however, more effective treatment strategies are needed to lower the burden from long-term use of glucocorticoids. A better understanding of the pathogenesis and clinical phenotypes of GCA will facilitate the identification of new targeted therapies that can provide safe, sustained remission and prevent disease relapse (BOX 1).

In this Review, we discuss the challenges encountered in studying the epidemiology of GCA subsets, the emergence of novel imaging techniques and their role in the diagnosis, monitoring and outcome prediction of GCA and PMR. Furthermore, we present a summary of our current understanding of the pathogenesis of GCA and the possible role of novel drugs in the treatment of GCA and PMR.

GCA and PMR: frequency and epidemiology

PMR is considered to be the second most common rheumatic disease in the elderly and, in countries where GCA is known to occur, GCA is the most frequent primary vasculitis[24,25]. The epidemiology of these conditions is challenging to study because of their common clinical and subclinical overlap. Large-scale epidemiological studies of GCA and PMR are lacking in several parts of the world, including Latin America, South Asia and Africa. The highest incidence of GCA and PMR is seen among populations of Northern European ancestry (and particularly in individuals of Scandinavian descent) in which the incidence of GCA and PMR, respectively, ranges from 18 to 29 and from 41 to 113 cases per 100,000 among people aged ≥50 years[25–32]. It is likely that the occurrence of GCA worldwide will increase due to ageing of the population. Indeed, the projected worldwide disease burden of GCA by 2050 is >3 million, and ~500,000 people will be visually impaired owing to GCA by 2050 (REF. 33).

Features of PMR are observed in 40–60% of patients with GCA at the time of diagnosis, and 16–21% of patients with PMR have GCA[1]. Subclinical GCA in patients with PMR can be detected by vascular imaging, but such imaging is not commonly performed in patients who seem to have PMR alone. Another difficulty is that there are no definite diagnostic tests for PMR and, even for GCA, the gold standard diagnostic test of temporal artery biopsy (TAB) is positive in only 39–87% of cases, and in <60% of patients with predominant LV-GCA[34–39].

LV-GCA occurs in up to 83% of patients with GCA and with unknown frequency in PMR. GCA may coexist in patients with PMR that have an incomplete response to glucocorticoids, constitutional symptoms and markedly elevated levels of acute phase reactants[40,41]. LV-GCA can be present when GCA is diagnosed or can occur at any point during the disease course, and it is detected with increasing frequency in patients with GCA after 4–5 years of disease[42]. The definition of LV-GCA is still imprecise, which hinders epidemiological studies. As biopsy of larger arteries is not feasible in routine practice, LV-GCA is diagnosed by use of imaging methods such as axillary ultrasonography, ^{18}F-FDG–PET, CT angiography (CTA) or MRI. All of these techniques assess mural inflammation and ultrasonography, CTA and MRI additionally investigate changes in the lumen.

Our revised understanding of GCA as a complex disease that is not limited to cranial arteries, along with advanced imaging techniques and international efforts to better define the disease, will facilitate future studies on the epidemiology of GCA and our understanding of its incidence, prevalence and disease course.

The role of imaging in GCA and PMR

Although imaging in GCA and PMR is evolving quickly, controversy persists concerning which imaging techniques to use when, and whether imaging is a reliable outcome parameter and/or tool for monitoring GCA and PMR.

Imaging techniques for diagnosing GCA and PMR. The majority of imaging studies have been performed to assess the potential of ultrasonography in diagnosing GCA and PMR. In GCA, ultrasonography has not yet replaced TAB, although several studies report a sensitivity of 55–100% and a specificity of 78–100% in the ability of ultrasonography to detect a 'halo' sign, which is a non-compressible

Box 1 | **Unmet needs in giant cell arteritis and polymyalgia rheumatic**

There are still many unmet needs in the clinical assessment and treatment of giant cell arteritis (GCA) and polymyalgia rheumatic (PMR). The top three unmet needs of different aspects of GCA and PMR are listed here.

Epidemiology

- Epidemiology and the extent of overlap between the GCA phenotypes of cranial GCA, large-vessel GCA (LV-GCA) and PMR
- Prevalence of subclinical GCA in clinically isolated PMR
- Expected increment of GCA and PMR incidence due to ageing of the population

Imaging

- Role of imaging methods in diagnosing GCA (as compared with temporal artery biopsy), in assessing large-vessel involvement in GCA and in diagnosing PMR
- Role of imaging in monitoring disease activity, predicting flares and assessing resulting large-vessel damage in GCA and PMR
- Role of evolving imaging techniques, such as contrast-enhanced ultrasonography, PET using novel tracers and optical coherence tomography–angiography in GCA and/or PMR

Etiopathogenesis

- Identify avoidable and treatable triggers for GCA and PMR
- Investigate the possibility that different disease pathways underlie GCA and lead to different clinical phenotypes, prognoses and treatment targets
- Interference with amplification and chronicity of inflammation and remodelling

Treatment

- Optimize the use of glucocorticoids and glucocorticoid-sparing agents
- Study novel treatment targets in GCA and PMR
- Define outcome parameters and identify prognostic factors for the stratification of treatment

hypo-echoic ring around the artery lumen reflecting inflammation of the vessel wall[1,35,43–45]. The TABUL study prospectively compared the performance of colour Doppler ultrasonography and TAB for the diagnosis of GCA, reporting sensitivities of 54% and 39% and specificities of 81% and 100%, respectively. As TAB was part of the reference standard, the higher specificity of TAB could be an artefact of the study methodology[36].

Although PMR is diagnosed clinically, ultrasonography can improve the accuracy of diagnosis and it has therefore been included in the ACR–EULAR classification criteria for PMR[46,47]. A characteristic lesion in PMR that can be detected by use of ultrasonography is subacromial bursitis or subdeltoid bursitis; the sonographic detection of these lesions diagnoses PMR with a sensitivity of 79% and a specificity of only 59%[48]. This low specificity relates to a true overlap between PMR and inflammatory arthritis as ultrasonography was better at distinguishing PMR from non-inflammatory mimics[46,47].

^{18}F-FDG–PET can establish a diagnosis of GCA in patients presenting with marked systemic symptoms and/or elevated levels of inflammatory markers without characteristic features of cranial GCA, and it can also be used to search for alternative diagnoses in patients with unexplained illness and a low probability of GCA[49]. ^{18}F-FDG–PET visualizes local glucose metabolism; as vascular inflammation is associated with increased glucose consumption, enhanced tracer uptake in the vessel wall suggests vasculitis. In patients with PMR, ^{18}F-FDG–PET has revealed increased glucose metabolism in the shoulder and hip girdle as well as the presence of lumbar interspinous bursitis and cervical interspinous bursitis[50,51]. One study also reported bilateral uptake of tracer in the fibrous capsule at the knees in 84% of patients with PMR[52]. ^{18}F-FDG–PET has also revealed that LV-GCA is present in up to 30% of individuals with PMR and is more likely to be present in patients who have PMR with anaemia, markedly elevated inflammatory markers and disease that is relapsing or resistant to treatment than in patients who have (treatable) PMR alone[51,53–55].

CTA and magnetic resonance angiography (MRA) enable the detection, in GCA, of soft tissue swelling or cuffing of the wall of large arteries and of the aorta, and also provide information about the luminal anatomy and blood flow. These techniques are thus helpful for detecting GCA-related vascular stenosis or aneurysms[56,57], although the sensitivity and specificity of these techniques for establishing a diagnosis of GCA is still unclear.

The role of high-resolution (3 Tesla (3T) and 7T) MRI in the investigation of cranial arteries in GCA is evolving. A multicentre trial comparing the ability of MRI (1.5T used in 55 patients and 3T in 130 patients) and TAB to detect cranial vasculitis in patients suspected to have GCA demonstrated a sensitivity for MRI (pooled analysis for 1.5T and 3T) of 88% and a specificity of 75%[58]. Exciting preliminary data suggests that new generation 7T MRI is even more sensitive than 3T MRI for detailing the segments of the temporal artery that are inflamed in GCA[59]. High-resolution MRI might also detect inflammation in both the deep temporal arteries and temporalis muscle, which is useful in patients where GCA is strongly suspected but the superficial temporal arteries appear normal[60]. The limited availability of 3T MRI and 7T MRI, however, restricts the clinical utility of this imaging modality in diagnosing and monitoring GCA.

Another pilot study of 12 patients with GCA or Takayasu arteritis observed that use of ^{18}F-FDG–PET with MRI had better soft tissue resolution and was optimal for determining disease extent for both diseases as compared with ^{18}F-FDG–PET with CT[61]. The performance of these imaging techniques was comparable when assessing the aorta and large vessels in GCA and Takayasu arteritis.

The value of imaging in monitoring GCA and PMR. The value of ultrasonography for assessing inflammation at temporal arteries is limited because the halo sign that is characteristic of GCA disappears after 2–4 weeks of glucocorticoid treatment and re-appears only in cases of major relapse[44,62–64]. Whether the extent, persistence or re-appearance of the halo sign at the temporal arteries is of any prognostic value for patients with GCA requires further investigation. Ultrasonography of large arteries such as the carotids or the axillary artery might be more useful for monitoring disease because wall swelling in these larger arteries persists longer than in superficial cranial arteries despite therapy[43]. Changes in the artery intima and media thickness during follow-up ultrasonography might reflect alterations of disease activity, but prospective evaluation is still needed to establish the importance of these changes[65].

^{18}F-FDG–PET, which is typically performed with CT, exposes patients to a radiation dose of 10–15 μSv, which precludes its routine use in follow-up investigations. Moreover, there is also uncertainty concerning the relationship between low-grade FDG uptake and arterial wall inflammation. In one prospective study, the level of arterial FDG uptake decreased following 3 months of glucocorticoid treatment. as compared with the level of uptake observed at the time of GCA diagnosis; however, no further reduction in uptake was seen at 6 months post-treatment despite clinical remission of GCA[40]. FDG uptake at 6 months post-treatment could reflect persistent arterial wall inflammation but it might also represent myofibroblast proliferation, fibrosis or the presence of atheroma, all of which consume glucose.

Longitudinal follow-up studies using MRA and CTA in GCA are scarce. In a prospective study, CTA scans were scheduled in 35 biopsy-proven cases at diagnosis and after 1 year of glucocorticoid treatment. Although arterial wall thickening was still present in 68% of cases after 1 year of treatment, the number of affected arterial segments, arterial wall thickness and contrast enhancement in the artery had decreased with therapy. No patients developed worsening of, or new, aortic dilation after 1 year of treatment, suggesting that aneurysm formation is a delayed complication in most patients with GCA[66]. This observation is in accordance with previous retrospective studies[42,67,68]. Lack of radiation exposure with MRI, as well as the option to use gadolinium-based contrast agents to distinguish active arterial wall inflammation from fibrosis, makes MRA an attractive tool for the follow-up of GCA, particularly in patients with LV-GCA[69]. The lower spatial resolution and longer scan times of magnetic resonance compared with CTA, and the infrequent but serious nephrotoxicity of gadolinium, are disadvantages of MRA.

No consensus exists regarding the screening for stenosis and aneurysms of large arteries and the aorta. Researchers attempt to obtain a baseline image of large arteries and the aorta in all patients with GCA (this information is especially useful in the assessment of patients with symptoms of LV-GCA), although there are no data proving the cost-effectiveness of this approach. Patients with an aortic diameter outside the sex and age-matched normal range and those with active aortitis or risk factors for the development of aortic aneurysms (for example, smokers, patients with hypertension and patients with pre-existing cardiovascular disease) might then be followed-up every 1–2 years with MRA to detect possible aortic dilatation while minimizing radiation exposure[70,71]. In patients that do not meet these criteria, axillary artery ultrasonography, chest radiography, echocardiogram and abdominal sonography every other year might be sufficient for monitoring disease progression, with any observed change in aortic diameter prompting further investigation[22].

Emerging developments in the imaging of GCA and PMR. The ability to reliably detect low-grade 'grumbling' arterial wall inflammation and early disease relapse in patients with GCA who are receiving treatment is desirable. New approaches to achieve this goal include the search for novel, specific PET ligands. PK11195 binds specifically to translocator protein, which is highly expressed on activated neutrophils, monocytes and macrophages. [^{11}C]-PK11195 identified the five patients with active disease among 15 patients with GCA and Takayasu arteritis with high sensitivity[72]. A small study of patients with GCA and Takayasu arteritis comparing colour Doppler ultrasonography with microbubble contrast-enhanced ultrasonography reported that the latter is optimal for the assessment of arterial wall lesions and that it detects neovascularization[73]. Moreover, initial evidence suggests that contrast-enhansed ultrasonography can quantify disease activity in patients with GCA and Takayasu arteritis and monitor the response to treatment in carotid arteritis[74–76].

Ischaemia of the optic nerve head, retina and choroid must also be assessed in patients with GCA. Traditionally fluorescein and indocyanine green angiography have been used in such assessments but these techniques are invasive and patients are at risk of allergic reactions to the dyes. Optical coherence tomography (OCT) is a noninvasive interferometric optical mode of imaging that could be of particular use in differentiating non-arteritic AION from GCA-related AION[77]. OCT uses motion-contrast imaging to produce high-resolution volumetric blood flow information that enables visualization of the distinct retinal, chororetinal and choroidal capillary networks[78,79]. In GCA, this technique might be applied to identify patients at risk of visual loss.

Understanding the pathogenesis of GCA

The current concept of GCA is that of an immune-mediated disease of large vessels; cranial arteries and/or the aorta and its major branches are thought to be the most frequent targets of this disease. Inflammation starts in the adventitia and spreads to the inner layers of the vessel wall. Patients with isolated PMR or polymyalgic syndrome in association with elderly onset inflammatory rheumatic diseases, such as rheumatoid arthritis (RA), are affected by a systemic inflammatory syndrome in conjunction with bursitis and synovitis of shoulders, hips and the spine[6]. PMR in association with GCA is regarded by some as an early or aborted form of vasculitis where vascular inflammation is often limited to the adventitia and periadventitial small vessels[80]. The acute phase of GCA is mainly inflammatory, whereas the chronic stages are characterized by inflammation, degradation and repair mechanisms that collectively lead to structural changes of the vessel wall, ischaemic complications and aneurysm development (FIG. 1). TABLE 1 highlights areas of future research based on the current understanding of the pathogenesis of GCA and PMR and notes potential biomarkers and treatment targets.

The role of inflammation in the initiation of GCA. The trigger for the inflammatory cascade resulting in GCA is still unclear. The adventitia is an important site of immune surveillance and it is rich in dendritic cells (DCs) expressing Toll-like receptors (TLRs) and in macrophages[81]. In GCA, these cells become aberrantly activated via

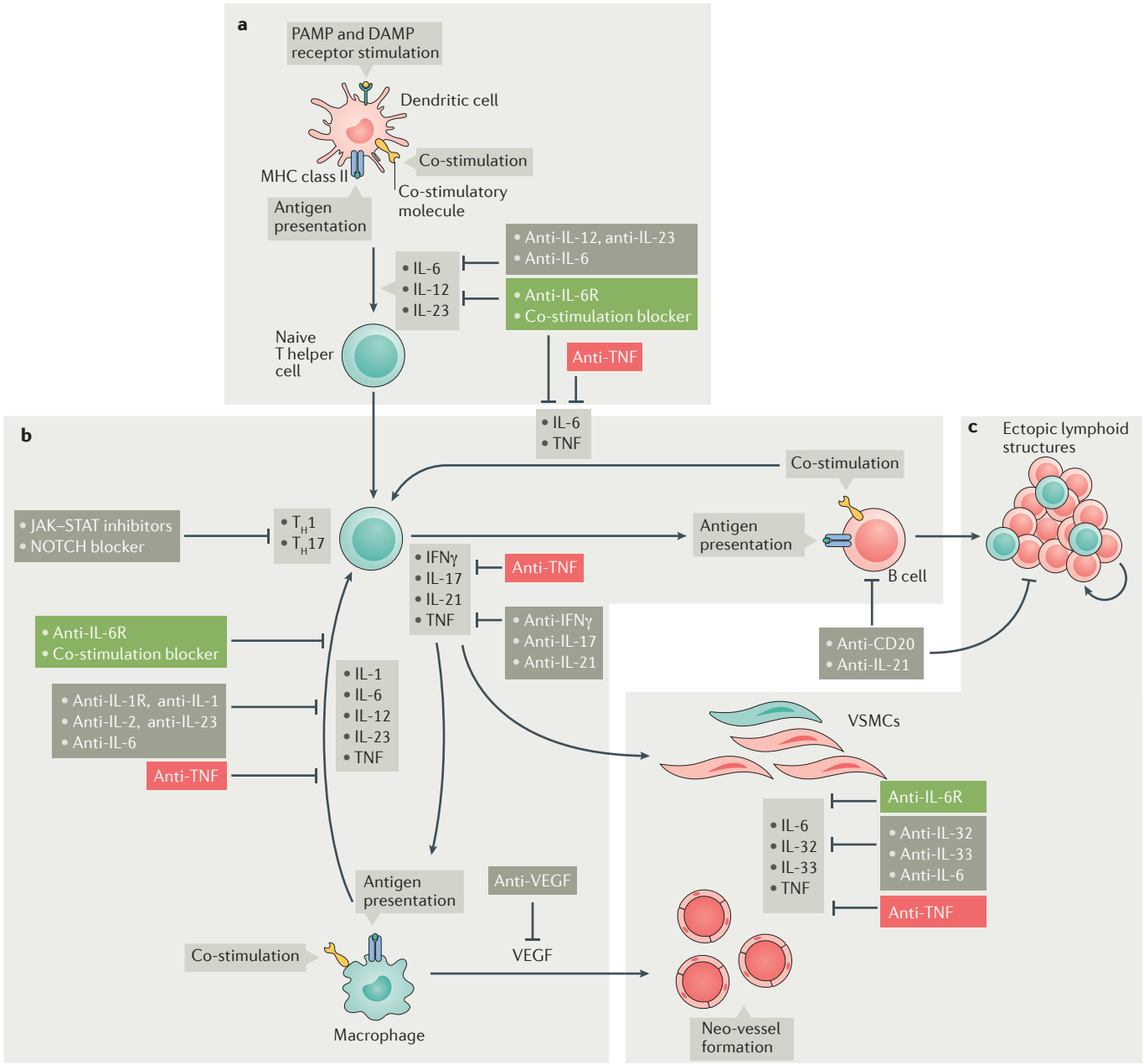

Figure 1 | **Pathogenetic pathways and treatment targets in giant cell arteritis. a** | During the initiation phase of giant cell arteritis (GCA), in which dendritic cells (DCs) within the adventitia are activated via pathogen-associated molecular patterns (PAMPs), microorganism-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs), pro-inflammatory cytokines such as IL-6, IL-12 and IL-23 are produced. In addition, naive T cells are activated via MHC class II molecules and the co-stimulatory molecules CD80/86 on the DCs that interact with the T cell receptor complex and CD28 present on T cells. **b** | Upon maturation of DCs and resident macrophages, naive $CD4^+$ T cells are stimulated to polarize into T helper 1 (T_H1) cells and T_H17 cells. Production of IFNγ and TNF by T_H1 cells and IL-17 and IL-21 by T_H17 cells enables these cells to recruit macrophages, which produce IL-1, IL-6, IL-12, IL-23, TNF and vascular endothelial growth factor (VEGF). Chemokines, which are produced by activated DCs and T cells, guide T cells, macrophages and B cells into the vessel wall. **c** | In the chronic phase of GCA, local hypoxia, together with the presence of macrophages and giant cells, amplifies the migration of both inflammatory cells and resident cells. In addition to cytokines (IL-6 and TNF) and chemokines, factors important in the chronic phase of GCA include VEGF, platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) produced by macrophages and endothelin-1 produced by endothelial cells and vascular smooth muscle cells (VSMCs), which activate inflammatory cells, VSMCs, stromal cells, pericytes and endothelial cells to induce the formation of new vessels and promote VSMC migration, fragmentation of the external and internal elastic lamina by metalloproteinases and endothelial cell proliferation. Ectopic lymphoid structures are formed within the adventitia in this phase of chronic inflammation and remodelling. Possible treatment approaches include prevention of DC activation in the initiation phase of GCA by use of antimicrobials, blockade of cytokines, chemokines, co-stimulatory pathways, Notch and signalling pathways by use of biologic and/or synthetic drugs in both the initiation and amplification phases of GCA, and blocking growth factors (including VEGF, PDGF and FGF), neurotrophins and cytokines in the chronic phase of GCA. Green boxes show treatments for GCA proven effective in RCTs; grey boxes show other potential treatment options for GCA; the red box shows a treatment that failed to show efficacy in clinical trials.

Table 1 | **Areas for future research in giant cell arteritis and polymyalgia rheumatica**

Pathogenetic mechanism	Matching clinical symptoms	Imaging findings	Possible biomarkers*	Possible treatment targets* (possible treatment)
Trigger or activation of innate immunity by activating DCs, T cells and B cells[81]	'Flu-like' symptoms, fever, night sweats	No known imaging technique	Acute-phase reactants, possibly IL-6	Infection (anti-infection therapies; immunization); DC activation (inhibition of IL-1 and IL-1β with canakinumab, gevokizumab or leflunomide); co-stimulation (abatacept); IL-6 (tocilizumab, sirukumab)
Arterial inflammatory infiltrate[91,108,150,151]	Headache, scalp pain, thickened arteries, painful arteries, constitutional symptoms	Characteristics of inflammation (e.g. 'halo' sign for GCA) found with sonography, MRA and/or PET	Acute phase reactants, neutrophilia	Co-stimulation (abatacept); JAKs (tofacitinib, baricitinib); cytokines such as IL-6, IFNγ, TNF, IL-12, IL-17 and IL-23 (cytokine blockers)
Vascular smooth muscle cell and intimal proliferation[93]	Jaw claudication, scalp or tongue necrosis, visual and other ischemic manifestations	Signs of inflammation and damage such as the 'halo' sign, occlusion and stenosis; changes in retinal and choroidal vessels as well as optic nerve head revealed by OCT angiography	Vascular biomarkers	VEGF (anti-VEGF); IFNγ (anti-IFNγ); IL-17 (secukinumab); neurotrophins (potential inhibitors of neurotrophins); endothelin-1 (inhibitors of endothelin-1)
Neo-angiogenesis, possible haemorrhage of media[93,118]	Jaw claudication, ischaemic damage (e.g. AION), scalp and tongue necrosis, aortic dissection	Increased axillary and carotid thickness of media, occlusion, stenosis and mural enhancement on contrast-enhanced ultrasonography	Markers of angiogenesis (e.g. elevated VEGF)	VEGF (anti-VEGF); proteases (inhibitors of MMPs, cathepsins, elastase)
Cytokine production, IL-6 (REF. 167)	Constitutional symptoms, polymyalgia	Main imaging findings in polymyalgia rheumatica are subdeltoid bursitis, bicipital tenosynovitis	Acute phase reactants	IL-6 (tocilizumab, sirukumab)
Medial thinning[90]	Aortic aneurysm	CTA, MRA to visualize aortic dilatation	Markers of vascular damage	No treatment targets identified except control of cardiovascular risk factors such as hypertension and dyslipidaemia
Fibrosis and chronic intimal-medial hyperplasia[94]	Large vessel stenosis, ischaemic limb pain	CTA, MRA to investigate luminal stenosis and/or occlusion	Markers of vascular repair	Vascular remodelling mediators such as MMP-9, TGFβ, PDGFA and PDGFB (inhibitors of vascular remodelling mediator proteins)

*This table intends to stimulate future research concerning various aspects of giant cell arteritis and polymyalgia rheumatica. It should not be understood as advice to apply all mentioned biomarkers and therapeutic interventions in clinical practice. AION, anterior ischaemic optic neuropathy; CTA, CT angiography; DC, dendritic cell; JAK, Janus kinase; MMP, matrix metalloproteinase; MRA, magnetic resonance angiography; OCT, optical coherence tomography; ^{18}F-FDG–PET, ^{18}F-fluorodeoxy-glucose PET; VEGF, vascular endothelial growth factor.

pathogen-associated molecular patterns (PAMPs) or microorganism-associated molecular patterns (MAMPs), leading to the production of pro-inflammatory cytokines such as IL-1 and IL-6 and the activation of T cells. A low level of expression of the co-inhibitory molecule programmed cell death 1 ligand 1 (PD-L1) by DCs in GCA seems to accelerate the recruitment and retention of T cells in the inflamed artery[82]. $CD8^+CCR7^+$ regulatory T cells with reduced expression of cytochrome *b*-245 heavy chain (also known as NADPH oxidase 2 (NOX2)) were detected in the peripheral blood of patients with GCA, resulting in decreased suppression of $CD4^+$ T cell responses[83]. In healthy individuals, $CD8^+CCR7^+$ cells release NOX2-containing vesicles that are taken up by interacting $CD4^+$ T cells, thereby inhibiting their activation, and T cells, macrophages and other immune cells eventually cause tissue damage in the media and adventitia and the release of damage-associated molecular patterns (DAMPs). DAMPs are increased in aged vessels where they act synergistically with PAMPs to further stimulate the inflammatory process[84].

Using advanced DNA sequencing techniques, one study observed abundant viral and bacterial DNA in the arterial wall of patients with GCA[85]. Earlier studies recurrently observed various bacterial strains (such as chlamydia and Burkholderia) or viruses (such as parvovirus B19 and varicella zoster virus) in temporal arteries, collectively supporting the hypothesis that PAMPs and MAMPs are crucial for the onset of GCA. No specific GCA-causing microorganism that might be targeted by anti-infective agents has been identified[86–88].

Treatment strategies directed at silencing DCs and adventitial macrophages at an early stage include the inhibition of IL-1 and IL-1β by drugs such as

canakinumab or gevokizumab and the blockade of T cell co-stimulation by abatacept. Some of these drugs have already been tested in GCA and/or PMR (see below).

Amplifying inflammation: feed-forward loops in GCA. Upon the maturation of DCs and resident macrophages, $CD4^+$ T cells are stimulated to polarize into T_H1 and T_H17 cells, which migrate in response to the chemokines that activate CXCR3 and CCR6 receptors[89]. T_H1 and T_H17 cells attract new macrophages via the production of IFNγ and IL-17, respectively[90]. In addition to $CD4^+$ T cells, a small proportion of $CD8^+$ T cells also infiltrates into the temporal artery in response to signals from CXCR3, producing IL-17 and IFNγ as well as perforin 1 and granzyme B. The level of perforin 1 and granzyme B produced seems to correlate with the extent of vessel wall destruction and disease severity[89]. IL-17 and IL-6 also regulate the crosstalk between T cells and a newly discovered subset of neutrophil granulocytes. In one study, $AnxA1^{hi}CD62L^{lo}CD11b^{hi}$ neutrophils were detected early after the initiation of glucocorticoid therapy and they suppressed T cell activity. Reducing the dose of glucocorticoids led to a rise in the level of IL-17 and IL-6, which changed the neutrophil phenotype to $AnxA1^{hi}CD62L^{hi}CD11b^{hi}$; these neutrophils were unable to control T cell responses[91]. Cytokine and chemokine gradients also orchestrate the migration of tissue-destructive monocytes, macrophages and B cells to amplify inflammation following the onset of GCA and, in parallel, neoangiogenesis is stimulated by VEGF that is released mainly by macrophages[92,93]. Targeting IL-6 receptor with tocilizumab and IL-6 with sirukumab, and blocking IL-17 with secukinumab or IL-12 and IL-23 with ustekinumab, as well as modulating chemokines or intracellular signalling pathways such as the Janus kinase–signal transducers and activators of transcription (JAK–STAT) pathway with inhibitors, might interrupt the feed-forward loops and terminate the amplification of inflammation.

IL-6 has a pivotal role in the pathogenesis of the systemic inflammatory response, whereas the recruitment of media-infiltrating macrophages, giant cell formation and the proliferation of VSMCs in GCA might also be driven by pro-inflammatory factors such as TNF and IFNγ[89,93–96]. JAK family members form homodimers or heterodimers to mediate the signalling of different cytokines[97]. IFNγ signals though JAK1–JAK2 heterodimers whereas type II cytokine receptors such as those for IL-6 and IL-1 mainly signal through JAK1 homodimers[98,99]. The JAK inhibitor tofacitinib preferentially blocks signalling by cytokine receptors that are associated with JAK3 and/or JAK1 (REFS 100,101). Baracitinib has selectivity for JAK1 and JAK2 dependent cytokine receptors and thereby targets T_H1 and T_H17 cells[102]. JAK1 and JAK2 dependent cytokine receptors are not exclusively expressed on T_H1 and T_H17 cells but, after binding of IFNγ and IL-6, they phosphorylate STAT1 and STAT3, which activate T-bet (T_H1 transcription factor) and RORγt (T_H17 transcription factor). Apremilast, which is effective in psoriatic arthritis and Behçet syndrome[103,104], binds to the catalytic site of the phosphodiesterase 4 (PDE4) enzyme and blocks the degradation of cyclic AMP (cAMP)[105]; the increased levels of cAMP result in a reduction in T_H1, T_H2, and T_H17-mediated immune responses and in the production of IFNγ, TNF, IL-12, IL-17 and IL-23, all of which have an important role in the pathogenesis of GCA[106,107]. Whereas these are promising agents to halt the transmural and/or systemic inflammation in GCA or PMR, there are no reports of JAK inhibitors or apremilast for treating these diseases.

Although B cells are present in temporal artery tissue of patients with GCA, their role in the pathogenesis of GCA and PMR has only recently been explored[108]. Circulating B cell levels are decreased in patients newly diagnosed with GCA or PMR and these levels recover rapidly once remission is achieved[92]. One study described the occurrence of tertiary lymphoid organs in the medial vessel-wall layer of temporal arteries, in close proximity to high endothelial venules, in 60% of patients with GCA[109]. It is also known that B cells can function as antigen-presenting cells and that they could thus provide the co-stimulatory signals required for the clonal expansion of $CD4^+$ T cells[110].

Arterial remodelling and vascular occlusion in GCA. In GCA, several mediators contribute to intimal hyperplasia and vascular occlusion. Platelet-derived growth factor (PDGF), for example, caused arterial occlusion in cultured primary myointimal cells derived from the human temporal artery and also stimulated the production of angiogenic factors (such as angiogenin) and chemoattractants (such as CCL2)[111]. In temporal artery specimens from patients with GCA, macrophages producing PDGFA and PDGFB were located at the media–intima junction, particularly in cases with concentric intimal hyperplasia[94]. Neurotrophins are growth factors that mediate the differentiation and survival of neurons and vascular cells. In GCA, nerve growth factor (NGF; predominately expressed in adventitia and media), brain-derived neurotrophic factor (BDNF; media and intima) and the neurotrophin co-receptor sortilin (adventitia and intima) were overexpressed in different histological layers of temporal arteries. *In vitro*, NGF and BDNF promoted the proliferation of VSMCs and BDNF also facilitated the migration of temporal artery VSMCs. Sortilin amplified proliferation and migration of VSMCs, functioning as an intracellular protein transporter for immature neurotrophins and as a regulator of BDNF trafficking and release[112]. Endothelin-1 and endothelin B receptor are also expressed in GCA lesions, particularly on VSMCs and multinucleated giant cells[113]. Endothelins might contribute to the pathogenesis of GCA by promoting inflammation, increasing the sensitivity of the lesion to vasoconstriction, increasing VSMC proliferation and stimulating the migration of VSMCs towards the intimal layer; these events collectively contribute to intimal hyperplasia and vascular occlusion[114–116].

Macrophages and giant cells from patients with GCA release VEGF; VEGF eventually leads to endothelial cell growth, neo-angiogenesis and vasa vasorum formation[93]. Whether local hypoxia (resulting from the high oxygen consumption of inflammatory and stromal cells) or

pro-inflammatory cytokines drive VEGF secretion needs to be elucidated[117]. Macrophages also produce proteases (including matrix metalloproteinases (MMPs), cathepsins and neutrophil elastase), which have an important role in the emergence, and branching, of vasa vasorum[118].

Whether vascular remodelling and intimal hyperplasia can be influenced by immunosuppressive therapies is unclear. One study obtained paired TABs from four patients (one treated with glucocorticoids, three with glucocorticoids and infliximab) at baseline and after 1 year of therapy, and reported that mRNA levels of inflammatory cytokines were decreased in arterial tissues whereas the mRNA levels corresponding to proteins that mediate vascular remodelling (such as MMP9, TGFβ, PDGFA and PDGFB) were increased[107]. Another study observed that tissue concentration of endothelin was similar in temporal artery specimens from patients with active disease and from patients with inactive (that is, treated) disease[116].

Further research is necessary to better understand the factors contributing to arterial remodelling and vascular occlusion in GCA and to determine how these processes can be interrupted. Although direct inhibitors of proliferative or pro-angiogenic factors hold promise, they could disrupt terminal arterial vascularization, such as of the vessels supplying the optic nerve, worsening ischaemic complications.

Improving the benefit of glucocorticoids

Glucocorticoids remain the mainstay treatment of both PMR and GCA, but the basis for the use of different glucocorticoid dosages in different clinical conditions is empiric[1]. According to consensus-based recommendations, the initial therapy is prednisone equivalent 12.5–25 mg per day for PMR and 40–60 mg per day for GCA, followed by individualized tapering regimens[20–22,119].

Optimizing the benefit:risk ratio of glucocorticoids to minimize adverse events while achieving sustained remission is an ongoing challenge[120]. Improved implementation of current treatment recommendations for the optimal use of glucocorticoids could reduce the burden of this treatment[20–22]. A EULAR task force concluded that the risk of glucocorticoid-related harm for the majority of patients taking glucocorticoids for a prolonged period (that is, for 3–6 months or more) is low if doses of ≤5 mg per day prednisone equivalent are prescribed, but high if doses >10 mg per day are used. At doses between 5 mg and 10 mg per day, patient-specific risk factors determine the probability of harm.

The development of innovative glucocorticoid preparations and/or glucocorticoid receptor ligands might also increase the benefit:risk ratio of glucocorticoids. A novel class of glucocorticoids are the dissociated agonists of the glucocorticoid receptors (DAGR; also known as selective glucocorticoid receptor modulators (SEGRMs))[121]. DAGRs predominately *trans*-repress products of glucocorticoid target genes that mediate anti-inflammatory effects without markedly transactivating the products of glucocorticoid target genes that are responsible for the adverse effects of these drugs[122]. Liposomal glucocorticoids have been designed to deliver conventional glucocorticoids to inflamed tissues using very small, nanometre-sized liposomes[123]. This technology could provide strong therapeutic effects and cause minimal systemic adverse events. DAGRs and liposomal glucocorticoids are currently being evaluated in RA; trials in PMR and GCA could follow if the results of these RA trials are favourable.

Finally, modified-release prednisone was shown to enable optimal chronotherapy with bedtime administration and the release of prednisone at the optimal time for suppression of pro-inflammatory cytokines (that is, at approximately 2 a.m.). Although modified-release prednisone was clinically superior to conventional prednisone in the treatment of RA (according to the CAPRA-1 and CAPRA-2 randomized controlled trials)[124,125], the multicentre randomized, phase III study in PMR was terminated early because of insufficient recruitment (only 62 of a planned 400 patients were included), which meant that this study failed to meet its primary endpoint[126]. Modified-release prednisone has also been studied in a small phase II trial including 12 patients with new-onset GCA. At 26 weeks, there was no difference between patients treated with modified-release prednisone as compared with patients treated with immediate-release prednisolone in terms of reduction in inflammatory markers, pain, fatigue and quality of life[127].

Emerging therapies for GCA and PMR

There is a need for glucocorticoid-sparing agents in the treatment of GCA and PMR. Methotrexate is currently the only conventional DMARD, if administered along with glucocorticoids, that demonstrates even a modest reduction of the cumulative glucocorticoid dose in systematic reviews and meta-analyses in GCA and PMR[23,128], although individual trials have reported that adjuvant methotrexate had no effect on the dose of glucocorticoid required to successfully treat GCA and PMR[129–131]. Current EULAR recommendations are conditionally in favour of using methotrexate in a subpopulation of patients with GCA and PMR[21,119]. For the use of other conventional DMARDs, such as azathioprine, mycophenolate mofetil, cyclophosphamide, ciclosporin or dapsone, in treating GCA and PMR there are either insufficient data from trials or the DMARD was ineffective or toxic in small, usually low quality, clinical studies[1]. Case series have shown some potential benefit of the DMARD leflunomide in patients with refractory GCA and PMR[132,133]; however, prospective evaluation of this drug in randomized controlled trials is still needed.

TNF antagonists were the first biologic agents studied in both GCA and PMR, either as monotherapy (in PMR) or in combination with glucocorticoids (in PMR and GCA). Initial case reports and case series revealed promising results; however, the results of randomized controlled trials of infliximab and etanercept (for the treatment of GCA and PMR) and of adalimumab (for the treatment of GCA) were disappointing[134–138] (TABLE 2). There is no clear explanation for the failure of these drugs, but there may be redundant pathways that render TNF blockade insufficient in treating these diseases.

Table 2 | **Clinical trials of biologic agents for the treatment of giant cell arteritis and polymyalgia rheumatica**

Agent (mechanism of action)	Trial information	Sample size (*n*)	Study population	Duration	Main results	Refs (publication type)
Polymyalgia rheumatica						
Infliximab (TNF blocker)	Randomized, multicentre, double-blinded	51	New, untreated PMR	52 weeks	Did not meet primary or main secondary end points	Salvarani 2007 (REF. 135) (full paper)
Etanercept (TNF blocker)	Randomized, single-centre, double-blinded	22	New, untreated PMR	14 days	Did not meet primary or main secondary end points	Kreiner 2010 (REF. 138) (full paper)
Tocilizumab (IL-6 receptor blocker)	Non-randomized, single-centre open-label	20	New PMR, treated with glucocorticoids ≤1 month	15 months	• Relapse-free remission off glucocorticoids at 6 months: 100% in tocilizumab group versus 0% in control group • Cumulative glucocorticoid dose: 1.1 g in tocilizumab versus 2.6 g in control group ($P = 0.01$) • Duration of glucocorticoid exposure: 3.9 months in tocilizumab group versus 14.1 months in control group ($P = 0.002$)	Lally 2016 (REF. 139) (full paper)
Tocilizumab (IL-6 receptor blocker)	Single group, multicentre open-label	20	PMR duration <12 months, glucocorticoid-naive or treated with glucocorticoids <1 month and off glucocorticoids for 7 days	24 weeks	• PMR-AS ≤10 at 12 weeks: 100%, no flares • Cumulative glucocorticoid dose: 0.8 g	Devauchelle-Pensec 2016 (REF. 140) (full paper)
Canakinumab; also known as ACZ885 (IL-1β blocker) or secukinumab; also known as AIN457 (IL-17 blocker)	Single-blind, randomized, three-arm proof-of-concept study	16	New PMR	2 weeks	Did not achieve primary end point	Matteson 2014 (REF. 146) (abstract, final report at ClinicalTrial.gov)
Giant cell arteritis						
Infliximab (TNF blocker)	Randomized, multicentre, double-blinded	44	New GCA (cranial)	54 weeks	Did not achieve primary and main secondary end points	Hoffman 2007 (REF. 134) (full paper)
Etanercept (TNF blocker)	Randomized, multicentre, double-blinded	17	GCA in remission, stable oral prednisone treatment	15 months	Cumulative glucocorticoid dose: 1.5 g in etanercept versus 3.0 g in control group ($p = 0.03$) other outcomes negative	Martinez-Taboada 2008 (REF. 137) (full paper)
Adalimumab (TNF blocker)	Randomized, multicentre, double-blinded	70	New GCA (cranial)	52 weeks	Did not achieve primary and main secondary endpoints	Seror 2014 (REF. 136) (full paper)
Tocilizumab (IL-6 receptor blocker)	Randomized, single-centre, double-blinded	30	New or relapsing GCA	52 weeks	• Complete remission at 12 weeks achieved in 85% of tocilizumab group versus 40% of the control group ($P = 0.03$); complete remission at 52 weeks achieved in 85% of tocilizumab group versus 20% of control group ($P = 0.001$) • Time to relapse: 50 weeks in tocilizumab group versus 25 weeks in control group ($P < 0.001$) • Discontinuation of glucocorticoids: 80% of tocilizumab group versus 20% of control group ($P = 0.004$) • Cumulative glucocorticoid dose: 43 mg/kg in tocilizumab group versus 110 mg/kg in control group ($P < 0.001$)	Villiger 2016 (REF. 18) (full paper)

Table 2 (cont.) | **Clinical trials of biologic agents for the treatment of giant cell arteritis and polymyalgia rheumatica**

Agent (mechanism of action)	Trial information	Sample size (*n*)	Study population	Duration	Main results	Refs (publication type)
Giant cell arteritis (cont.)						
Tocilizumab (IL-6 receptor blocker)	Randomized, multi-centre, double-blinded	251	GCA, new or refractory	52 weeks + 52-week open-label extension phase	• Sustained remission at 12 months: 56% (weekly injections) or 53% (bi-weekly injections) in the tocilizumab groups versus 14–18% in the control groups ($P < 0.0001$) • Cumulative glucocorticoid dose at 12 months: 1.8 g in both tocilizumab groups compared with 3.3 g and 3.8 g in the two placebo groups (which had short and long glucocorticoid tapering courses, respectively)	Stone 2016 (REF. 19) (full paper)
Sirukumab (IL-6 blocker)	Randomized, multi-centre, double-blinded	204	Active GCA	52 weeks + 52-week open-label extension phase	Results expected by end of 2018	NCT02531633 (ongoing study)
Abatacept (CTLA-4 Ig)	Randomized, multi-centre, double-blinded	49	New or relapsing GCA	12 months	Relapse-free remission at 12 months: 48% in abatacept versus 31% in control group ($p = 0.049$)	Langford 2015 (REF. 152) (full paper)
Abatacept (CTLA-4 Ig)	Randomized, multi-centre, double-blinded	98	Active GCA or Takayasu arteritis	48 months	Results not yet available	NCT00556439 (study completed)
Abatacept (CTLA-4 Ig)	Randomized, multi-centre, double-blinded	200	New GCA	52 weeks	Estimated completion by 2021	NCT03192969 (recruiting)

CTLA-4, cytotoxic T-lymphocyte protein 4; GCA, giant cell arteritis; PMR, polymyalgia rheumatica; PMR-AS, polymyalgia rheumatica activity score.

The results from recent trials of tocilizumab in GCA have generated optimism for this approach. A phase II 52-week study of 30 patients with GCA suggested that treatment with intravenous tocilizumab in combination with a short cycle of glucocorticoids resulted in higher remission rates, lower cumulative glucocorticoid doses, and a shorter duration of glucocorticoid therapy compared with placebo treatment[18].

In the phase III GiACTA trial, 119 newly diagnosed patients and 132 patients with relapsing GCA were randomly assigned to receive weekly or every-other-week subcutaneous tocilizumab in combination with a 26-week prednisone taper, or to one of two placebo arms in which prednisone was tapered over 26 or 52 weeks[19]. The primary outcome of sustained prednisone-free remission (defined as the absence of a disease flare and normal C-reactive protein (CRP) levels) at week 52, was achieved in 56% of the patient group that received tocilizumab weekly and in 53% of the patient group that received tocilizumab every other week. By contrast, only 14% of patients in the placebo arm in which prednisone was tapered over 26 weeks, and 18% of patients in the placebo arm in which prednisone was tapered over 52 weeks, achieved the end point. Because of the direct influence of tocilizumab on acute phase reactants, a sensitivity analysis was conducted excluding CRP levels from the definition of sustained remission. This analysis confirmed the primary results. Other outcomes, such as the proportion of patients with at least one flare or quality of life, were also better in the tocilizumab-treatment groups. The cumulative glucocorticoid dose was ≥40% lower in tocilizumab-treated patients than in glucocorticoid-treated patients and serious adverse events occurred in 14–15% of tocilizumab-treated patients compared with 22–26% of patients in the placebo groups. Whether the rate of serious adverse events and cumulative glucocorticoid dose were directly related is unclear, as the study was not powered to investigate such an association. Longer follow-up of patients treated with tocilizumab is now required to determine the durability of remission and the safety of tocilizumab. On the basis of the results of GiACTA and other trials, tocilizumab has been approved by the FDA for use in GCA[19].

In PMR, two prospective open-label studies of tocilizumab (one with accompanying glucocorticoids and one without; neither study included a proper control group) reported achieving the primary efficacy end point, namely low disease activity at 12 weeks, defined as PMR activity score (PMR-AS) ≤10, or glucocorticoid-free remission at 6 months, in 100% of patients[139,140]. The PMR-AS combines, into a quantitative score, the patient's assessment of pain and the physician's global assessment, both of which are assessed on a 0–10 visual analogue scale, with the duration of morning stiffness, the elevation of the upper limbs (a semi-quantitative assessment scored on a 0–3 scale) and CRP levels.

These studies complement the evidence from numerous case reports, case series and small non-randomized studies reporting a benefit of tocilizumab in PMR and GCA[141–144]. The data on PMR, however, are still insufficient to recommend tocilizumab treatment for this condition other than in trials or in exceptional cases, such as in glucocorticoid-resistant disease or when glucocorticoids are contraindicated.

A phase III study of the IL-6 blocker sirukumab or placebo plus glucocorticoids in GCA is ongoing. Recruitment is expected to be completed in the second half of 2017 and the first results could be available by the end of 2018 (REF. 145).

Continuing with other cytokine inhibitors, a proof-of-concept study assessed the efficacy in PMR of canakinumab, an IL-1β inhibitor, and secukinumab, an IL-17 inhibitor, in comparison with glucocorticoid therapy[146]. This trial failed to meet its primary end point but the observational period of 2 weeks might have been too short to demonstrate any notable effects. A study of the anti-IL-1β antibody gevokizumab in GCA was terminated early because of the negative outcome of a trial of this agent in Behçet disease[147,148].

Ustekinumab, which blocks IL-12 and IL-23, has been studied in a small open-label trial of patients with treatment-refractory GCA in which ustekinumab was given along with glucocorticoids[149]. A reduction in features of disease activity leading to reduction of the glucocorticoid dose as well as the possibility for discontinuing other immunosuppressive agents was reported[149]. The role of this agent for treatment of GCA outside of clinical trials remains to be defined.

Inhibiting T cell activation and halting the inflammatory cascades that lead to transmural inflammation by T cells and macrophages might halt the destruction of the arterial wall in GCA[150,151]. Blocking T cell co-stimulatory signals with abatacept was compared with placebo, in a small RCT in GCA which included glucocorticoids in both treatment arms[152]. A markedly higher rate of relapse-free remission was achieved after 12 months in the abatacept group compared with the placebo group. The majority of T cells in the arterial wall of patients with GCA, however, are effector cells lacking co-expression of CD28, the interaction of which with B7 molecules is usually targeted by abatacept[84]. This observation raises the intriguing possibility that the success of this study could relate to an off-target effect of abatacept in GCA. Interestingly, in Takayasu arteritis, abatacept was no more effective than placebo in maintaining remission[153].

A few cases of GCA have also been treated successfully with rituximab (a B-cell depleting monoclonal antibody therapy), suggesting that placebo-controlled trials might be warranted to better study this agent for its ability to maintain remission and to enable glucocorticoid-sparing in GCA[154,155].

The role for biologic DMARDs in treating GCA and PMR is emerging and these drugs could become routine clinical care in the near future. The successful use of biologics with subsequent rapid tapering of glucocorticoids could markedly reduce the burden of glucocorticoid-related adverse effects.

Future treatments for GCA and PMR

To ensure the optimal treatment of GCA and PMR in the future, it is important to determine which patients will benefit from treatment with biologic agents, how biologic agents can be used and what the best treatment targets for GCA and PMR are.

Which patients with GCA and PMR will benefit from biological agents? The current unmet clinical need in GCA and PMR is the treatment of patients with a persisting high burden of inflammatory disease, multiple relapses with an inability to wean glucocorticoids, non-response to methotrexate, co-morbidities and other factors that increase glucocorticoid-related adverse events, and resistance to glucocorticoid therapy[17,70,156–159]. It is anticipated that biologic agents, particularly IL-6 inhibitors, will first be used in these subpopulations even though clinical trials have focused on patients with new-onset or relapsing disease[18,139,140].

Biologic agents might also be used early in patients at risk of disease complications and/or treatment-related adverse events. Unfortunately, the majority of data on prognostic factors in PMR and GCA are weak or contradictory, impeding the definition and identification of the 'at risk' population[128] (C.D., B.D. and S. Gonzalez-Chiappe, unpublished work). A pronounced inflammatory response at disease outset has been associated with a higher probability of relapse in both GCA and PMR[15,16,160]. Assuming that IL-6 blockade would be particularly effective in cases with high levels of systemic inflammation, these patients could benefit most from treatment with IL-6 blockers.

How will biologic agents be used in GCA and PMR therapy? A rapid response to glucocorticoids has been considered an important feature in the treatment of GCA and PMR for decades. Immediate treatment and a rapid response is also pivotal to prevent blindness in GCA[12,13]. Although tocilizumab yielded impressive results in trials assessing its ability to maintain GCA remission, it is unclear if tocilizumab therapy without glucocorticoids will prevent vascular complications such as sight loss or aneurysms[18,19]. The outcome parameters used in these studies mostly reflected the inflammatory response rather than underlying vessel wall damage. In PMR, tocilizumab without glucocorticoids did not rapidly improve symptoms; although 100% of patients with PMR treated with tocilizumab achieved the primary end point, improvement was more gradual than that seen with glucocorticoids[140]. A low disease activity, as defined by the PMR-AS, was achieved by less than 50% of patients after 4 weeks.

The response of patients with GCA to abatacept might also be more gradual compared with the response induced by glucocorticoids; however, due its mode of action, the effect of abatacept might be more lasting and have a greater impact on reducing vascular damage than glucocorticoids[150].

Based on these results it is unlikely that biologics will be used as monotherapy to induce remission in GCA and PMR in the near future. Instead, a short course of glucocorticoids might be required to produce a rapid

improvement of symptoms, before remission is maintained by use of biologic therapies. It is unclear how long treatment with biologic agents needs to be continued once stable remission has been achieved; it might be possible to change to another agent, such as methotrexate, to maintain remission. Long-term treatment with biologics or other immunosuppressive agents might prevent late vascular complications in patients with LV-GCA, although this benefit still needs to be demonstrated in clinical studies.

What are the treatment targets in GCA and PMR? The targets for the treatment of GCA and PMR need to be more clearly defined. Whereas the remission of symptoms or the prevention of blindness are obvious treatment goals, other treatment goals — as elicited in in-depth discussions with patients as well as in patient surveys — are less clear[161,162]. For example, patients with PMR complain about disability and fatigue, symptoms that they rated as important as pain. 'Coming off steroids' as well as 'living with steroids' were also important to individuals according to a survey conducted by the GCA and PMR charity group GCAPMRuk (REF. 163). In addition, remission and relapse have been defined differently in the majority of published studies[164].

Studies in GCA have used qualitative criteria of remission and relapse as outcome measures, taking into account the history and clinical assessment of GCA and/or PMR features, the physician's global assessment, erythrocyte sedimentation rate (ESR), CRP levels, blood count and fibrinogen levels. Remission was classed as the absence of abnormal findings of these parameters whereas a relapse was considered if characteristic signs and symptoms of the disease reappeared[19,129,134]. The absence of a relapse does not automatically imply remission. The prognostic relevance of low-grade disease activity states, which are compatible with neither remission nor relapse, is currently unclear. Trials in PMR have used either the composite PMR-AS to define remission and low disease activity or have applied qualitative remission and relapse criteria[135,138,140,164,165].

Another challenge of outcome criteria for GCA and PMR is the fact that certain agents, such as IL-6 antagonists, directly influence acute phase reactants which are integral to current remission and relapse criteria[19,129,134,164,166]. The inclusion of ESR and CRP level in outcome measures of anti-IL-6 trials might therefore produce a type I error (that is, the incorrect rejection of a true null hypothesis); however, remission and relapse criteria without a laboratory criterion are unavailable. Whether imaging of vessels involved in GCA or joints and periarticular structures in PMR, or the use of biomarkers that are independent of the acute phase response, would be a possible alternative to ESR and CRP in the remission and relapse criteria is unclear.

Another unanswered question is whether treatment decisions could be based on abnormal imaging with or without laboratory results. It might be tempting to modify treatment in a patient with GCA who has elevated acute phase reactants and positive ^{18}F-FDG–PET despite the absence of symptoms[40]. We do not know, however, whether these tests are reliable markers of ongoing inflammation or even predictive of future large vessel damage and resultant complications, and whether patients would benefit if treatment was changed on the basis of these parameters.

Conclusions

The understanding that GCA and PMR have overlapping clinical phenotypes, new developments in the field of imaging as well as new treatment options have raised new questions and identified unmet needs in the diagnosis, treatment and prognostics of these diseases. First, what is the true epidemiology of these diseases given the frequent clinical and subclinical overlap of cranial and large vessel disease, and the overlap of GCA and PMR? Second, do different pathophysiological pathways determine the clinical phenotype, prognosis and treatment response of GCA and PMR? Third, which biomarkers can help physicians to recognize and predict unfavourable disease outcomes of GCA and PMR? Fourth, what is the role of currently available and evolving imaging techniques for diagnosing and monitoring GCA and PMR? Fifth, how can emerging therapies be used to treat GCA and PMR and, finally, what treatment targets should be used in future clinical studies of GCA and PMR? Multinational collaboration is needed in order to conduct studies that answer these questions.

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New insights into the epigenetics of inflammatory rheumatic diseases

Esteban Ballestar and Tianlu Li

Abstract | Over the past decade, awareness of the importance of epigenetic alterations in the pathogenesis of rheumatic diseases has grown in parallel with a general recognition of the fundamental role of epigenetics in the regulation of gene expression. Large-scale efforts to generate genome-wide maps of epigenetic modifications in different cell types, as well as in physiological and pathological contexts, illustrate the increasing recognition of the relevance of epigenetics. To date, although several reports have demonstrated the occurrence of epigenetic alterations in a wide range of inflammatory rheumatic conditions, epigenomic information is rarely used in a clinical setting. By contrast, several epigenetic biomarkers and treatments are currently in use for personalized therapies in patients with cancer. This Review highlights advances from the past 5 years in the field of epigenetics and their application to inflammatory rheumatic diseases, delineating the future lines of development for a rational use of epigenetic information in clinical settings and in personalized medicine. These advances include the identification of epipolymorphisms associated with clinical outcomes, DNA methylation as a contributor to disease susceptibility in rheumatic conditions, the discovery of novel epigenetic mechanisms that modulate disease susceptibility and the development of new epigenetic therapies.

Since the conceptual definition of epigenetics was developed by Conrad Waddington in 1942, great strides have been made towards understanding the importance of the addition, maintenance and removal of epigenetic modifications to DNA and histones for cellular function and identity (BOX 1). To mediate the correct expression (and repression) of distinct sets of genes, epigenetic marks not only act in tight coordination with other regulatory elements, such as transcription factors and non-coding RNAs, but are also connected with upstream signalling pathways and, in some cases, with extracellular factors. One relevant example of this coordination is the influence of cytokines and growth factors (present in the bone marrow, blood and tissues) on the acquisition of epigenetic marks that are associated with the terminal differentiation and activation of immune cells[1,2]. Given the importance of the epigenome in defining cell identity and ensuring normal cell function, a greater understanding of how aberrant changes in epigenetic profiles cause cellular dysregulation and disease is urgently needed.

In the past decade, epigenomic profiling has been crucial for discovering associations between epigenetic alterations and disease. These epigenetic alterations are caused by several mechanisms, including genetic mutations in epigenetic factors that are directly or indirectly responsible for the acquisition of epigenetic marks[3]. Moreover, certain single nucleotide polymorphisms (SNPs) conferring disease susceptibility can markedly alter the acquisition of epigenetic marks and thereby alter expression profiles[4] (FIG. 1). For example, a study published in 2016 showed that SNPs can have long-distance effects as they alter the function of distal regulatory elements to mediate gene expression changes[5]. Finally, exposure to certain environmental factors can mediate the occurrence of aberrant epigenetic profiles, influencing gene expression and ultimately compromising the function of cells[6].

The complex aetiology of inflammatory rheumatic diseases, in which interplay between genetic predisposition and environmental factors contributes to disease development, makes their study especially challenging. Studies in twins have been particularly valuable in determining the role of epigenetics in the development of rheumatic diseases in addition to genetic determinants; for instance, monozygotic twin discordance, where one twin develops the disease while the other remains healthy, further highlights the complexity of inflammatory rheumatic diseases[7,8]. Early genetic studies revealed a causative association between genes encoding HLA

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Key points

- Epigenetic mechanisms are essential for immune cell differentiation and function, including the correct activation of B cells and T cells and inflammatory processes
- The dysregulation of epigenetic mechanisms in genetically predisposed individuals is associated with inflammatory rheumatic diseases
- Epigenome-wide association studies in genetically complex inflammatory rheumatic diseases have identified substantial correlations between epigenetic mechanisms and disease activity and severity
- Epigenetic dysregulation contributes to the clinical manifestations of monogenic autoinflammatory syndromes and can be used as a biomarker of response to treatment
- The systematic use of epigenomic screening will help to classify and identify novel biomarkers for personalized management of patients with inflammatory rheumatic diseases
- New inhibitors of epigenetic enzymes or upstream enzymes that are linked to the epigenetic control of immune function are likely to be tested in clinical trials for disease management

molecules and the development of several autoimmune and autoinflammatory diseases. Currently, many genome-wide association studies (GWAS) have identified variants of immune-related genes that confer susceptibility to such diseases. Furthermore, several studies have linked epigenomic alterations in innate and adaptive immune cells (resulting in altered cell function) to the acquisition of disease phenotypes, including those in rheumatic autoimmune diseases such as rheumatoid arthritis (RA)[8], and in autoinflammatory diseases such as cryopyrin-associated periodic syndromes (CAPS)[9]. These findings reinforce the importance of epigenetics in the development of inflammatory rheumatic diseases.

In this Review, we provide an up-to-date overview of the epigenetic mechanisms involved in immune cell function, and discuss how their dysregulation contributes to genetically complex autoimmune inflammatory diseases such as RA and systemic lupus erythematosus (SLE), as well as monogenic diseases including CAPS and familial Mediterranean fever (FMF). Finally, we discuss how our growing knowledge of the role of epigenetics in disease development can aid the discovery of novel biomarkers and therapeutic targets, with the goal of providing personalized treatments.

Epigenetic mechanisms in immune cells

Immune cells need to respond rapidly to a variety of environmental stimuli. Epigenetic mechanisms are crucial for the differentiation of haematopoietic cells as well as for providing the plasticity required by these cells for fast and efficient functional responses. Adaptive and innate immune cells form two divergent branches of the immune system and have substantial differences in the elements involved in epigenetic control. Some of the main differences are related to the epigenetic regulation of sets of transcription factors that are expressed in the lymphoid and myeloid branches of the immune system during haematopoiesis; these differences become more marked as immune cells differentiate along each branch. Genome-wide epigenetic studies have shown that during the differentiation of haematopoietic stem cells (HSCs), both DNA methylation and histone modifications have pivotal roles in the transition from a transcriptional programme favouring pluripotency and proliferation, to one that determines final cell identity and function[10]. Lineage-specific transcription factors, including C/EBPα, GATA3, EBF1 and PAX5, which are methylated in HSCs and have both repressing and activating (bivalent) histone marks[11], are demethylated in a lineage-specific and stage-specific manner[12]. These cell type-specific methylation changes are linked to specific chromatin signatures that determine cell identity. For example, in certain genomic regions of terminally differentiated myeloid cells, the level of monomethylated histone H3 lysine 4 (H3K4me1) is higher than in the corresponding genomic regions of their progenitors, whereas the opposite is true in lymphoid cells[12]. Furthermore, the amount of acetylated histone H3 lysine 27 (H3K27ac) is consistently higher in lymphoid cells than in myeloid cells, again highlighting how differences in the epigenetic landscapes of each haematopoietic cell lineage determine the terminal differentiation of these cells[12].

Other differences between myeloid and lymphoid lineage-specific epigenetic changes are related to the biology of the innate and adaptive immune systems. One such example is the activation of naive B cells and T cells upon exposure to antigens. Whole genome bisulfite sequencing of B cells and T cells revealed striking differences in the levels and distribution of epigenetic marks during the transition from naive to effector cells[13,14]. Lineage commitment of $CD4^+$ and $CD8^+$ T cells requires a complex interplay between DNA methyltransferases (DNMTs) and specific chromatin sites. This notion was highlighted by a study reporting the identification of a differentially methylated region near the *CD4* gene in different T cell populations; this region was hypermethylated in double-negative and double-positive thymocytes as well as in mature cytotoxic $CD8^+$ T cells, whereas hypomethylation was observed in $CD4^+$ T cells[13]. Another study showed that the lineage-specific, transcription factor-dependent recruitment of the methylcytosine dioxygenase TET2 to genomic regions containing 5-hydroxymethylcytosine is crucial to $CD4^+$ T cell differentiation[15].

DNA methylome analysis of B cells across distinct differentiation stages showed that their activation is dominated by hypomethylation, whereas memory B cells maintain a stable methylation signature to enable the rapid reactivation of plasma cells[14]. In contrast to lymphocytes, which are dependent on exposure to specific antigens to become activated, myeloid cells respond to a wide range of stimuli, including bacterial antigens, cytokines and factors that are present in the inflamed tissues to which they are recruited. In this respect, innate immune cells require a high degree of plasticity to respond rapidly and adequately to a wide range of stimuli; such plasticity is achieved through the interdependent coordination of several epigenetic mechanisms mediating gene expression. Upon activation of macrophages and dendritic cells, drastic global gene expression changes occur rapidly: many of the

Box 1 | **Overview of epigenetic mechanisms**

Epigenetic mechanisms include the covalent modifications of DNA and histones. Chemical modifications to DNA, which are catalysed by DNA methyltransferases, consist of the methylation of cytosines followed by guanines (CpG sites). 5-Methylcytosines can undergo subsequent ten-eleven translocation (TET) enzyme-mediated oxidation of methylcytosine to yield 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxycytosine. These oxidized forms of cytosine are considered intermediates of demethylation, as they can be removed enzymatically, leading to the restoration of the unmethylated form of cytosine. However, these oxidised forms can be stable epigenetic marks. In mammals, adenines can also be methylated, and this epigenetic mark is also associated with transcriptional regulation. Studies of adenine methylation and its role in the regulation of gene expression are expected to grow exponentially over the next few years. With regard to histone modifications, well-studied roles in gene regulation include those associated with changes in promoter regions, mainly modifications such as trimethylation of histone H3 lysine 4 (H3K4me3) or trimethylation of histone H3 lysine 27 (H3K27me3), as well as different acetylated forms of histones H3 and H4. Over the past 6 years, the role of histone modifications in enhancer regions has been studied in depth. Modifications that have a functional role in such regions include monomethylation of histone H3 lysine 4 (H3K4me1) and acetylation of histone H3 lysine 27 (H3K27ac).

regulated genes undergo a previous DNA demethylation step that is coupled with changes in histone marks[16]. The terminal differentiation of monocytes into different cell types, including pro-inflammatory and anti-inflammatory macrophages, osteoclasts and dendritic cells, is accompanied by further global epigenetic changes. Key transcription factors that mediate the terminal differentiation of myeloid cells interact closely with epigenetic modifiers to ensure the acquisition of appropriate epigenetic marks conferring cell identity. For instance, transcription factor PU.1 interacts with and recruits DNMT3B and TET2 to mediate DNA methylation changes at specific genomic sites during the terminal differentiation of monocytes[17]. Also, signal transducer and activator of transcription 6 (STAT6) is able to direct specific DNA methylation changes downstream of IL-4, which are responsible for the acquisition of dendritic cell identity[16].

Another relevant example of how the biology of innate immune cells is regulated by epigenetic mechanisms is the ability of these cells to respond to insults such as bacterial infections. In some cases, innate immune cells develop an enhanced response to infections, a process commonly known as 'training'. In other circumstances, innate immune cells develop a diminished response, known as endotoxin tolerance. The development of innate memory is especially relevant to inflammation, as inappropriate responses can lead to disease. Re-stimulation of monocytes trained with β-glucan, a major cell wall component of the yeast *Candida albicans*, leads to an enhanced production of cytokines such as IL-1β, which is dependent on the mechanistic target of rapamycin (mTOR)–hypoxia-inducible factor 1α (HIF1α) signalling pathway. The genes specifically induced or repressed by β-glucan display dynamic changes in histone modifications[18]. In particular, distal regulatory elements that gained H3K27ac marks also gained H3K4me1 marks, although the H3K4me1 marks remained even after the loss of H3K27ac marks[19], suggesting that H3K4me1 acts as a key mark that confers epigenetic memory, so that, upon re-stimulus, genomic regions containing H3K4me1 marks are quickly recognized and assigned to regain H3K27ac marks for rapid and enhanced transcriptional response[19]. In contrast to trained monocytes, lipopolysaccharide (LPS)-tolerized monocytes persistently accumulate H3K4me1 marks in genomic regions corresponding to enhancers involved in cytokine responses and nuclear factor-κB (NF-κB) signalling. Upon LPS re-stimulation, the deposition of H3K27ac to these H3K4me1-marked regions is reduced or blocked to diminish monocyte responses[20].

Given the crucial role of epigenetics in the differentiation, activation and inflammatory responses of immune cells, epigenetic alterations have a huge effect on both the phenotype and biology of inflammatory diseases.

Epigenetics and inflammatory diseases

Whereas transient inflammation protects tissues from pathogenic invasions, inappropriate inflammation can cause a wide spectrum of diseases characterized by genetic and epigenetic risk factors. The concept of epigenetic variability in the context of physiological and pathological functions of immune cells is complex. A main distinction of the effect of epigenetic changes in inflammatory diseases can be made between monogenic and genetically complex diseases. Some monogenic diseases, for example CAPS[9], can either result from a single genetic defect, which can be directly or indirectly linked to certain downstream epigenetic changes, or can be associated with a range of different clinical symptoms depending on the existing epigenetic status of individual patients. In the case of genetically complex diseases, such as SLE and RA, different genetic susceptibility variants can be linked to a range of downstream epigenetic alterations, increasing the genetic complexity of the disease. Therefore, it is necessary to assess the biology of each disease to define the contribution of factors surrounding epigenetic alterations and to understand how these alterations result in the specific aetiologies. Over the past 5 years, numerous studies have demonstrated the widespread occurrence of epigenetic alterations in both genetically complex and monogenic inflammatory rheumatic diseases (TABLE 1).

Epigenome-wide association studies and inflammatory disease risk. GWAS have identified many polymorphisms associated with susceptibility to different types of genetically complex autoimmune diseases such as RA, SLE, systemic sclerosis and Sjögren syndrome. These genetic variants can cause cell-specific effects and result in defective immune cell populations. For example, polymorphisms in *NOD2*, a gene associated with Crohn's disease that encodes an intracellular receptor for bacterial peptidoglycans in the monocytic lineage, cause hyperresponsiveness to NOD-like receptor ligands and Toll-like receptor (TLR) ligands[21,22]. Furthermore, GWAS have shown a shared genetic susceptibility across a wide range of diseases that affects related tissues. This overlap is remarkable in immune-related diseases, which

include disorders showing similar clinical manifestations[23]. One of the first associations identified was that of the Arg381Gln (rs11209026) variant of the *IL23R* gene with several inflammatory diseases, including psoriasis, RA and multiple sclerosis[24,25]. Given the important role of IL-23 receptor in the polarization and activation of T cells, the frequency of this variant also highlights the causal contribution of T cell dysfunction to disease[26].

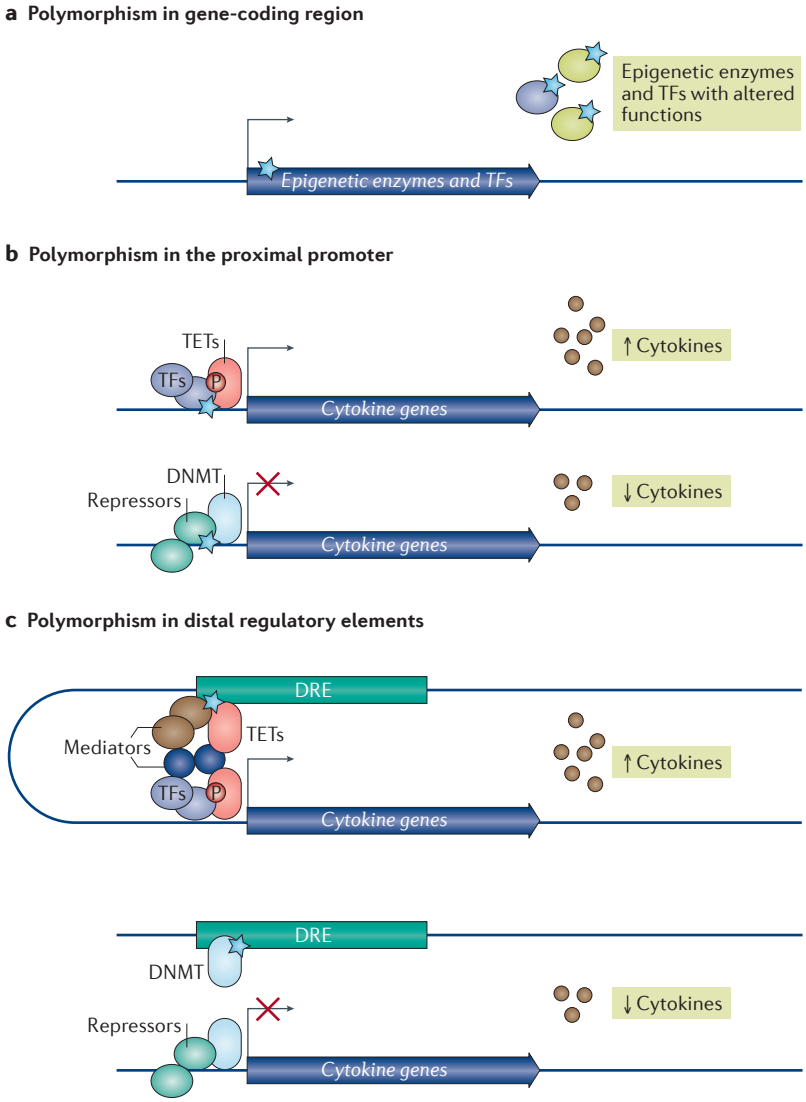


Figure 1 | **Potential links between genetic polymorphisms and epigenetic changes in inflammatory rheumatic diseases. a** | Polymorphisms occurring in the coding regions of genes encoding epigenetic enzymes or transcription factors (TFs) can give rise to aberrant enzymes or TFs that mediate adverse chromatin modifications in distal genes. **b** | Polymorphisms in proximal promoters might affect the recruitment of TFs and epigenetic enzymes, such as ten-eleven translocation enzymes (TETs) or DNA methyltransferases (DNMTs), which might lead to altered cytokine expression. **c** | Polymorphisms occurring at a distal regulatory element (DRE) can result in aberrant DNA methylation of this region. DREs can recruit mediators that in turn recruit TFs and TETs to distant promoters, thus altering the transcription of distal genes. Conversely, these polymorphisms can also reduce gene transcription *in trans* by preventing the recruitment of mediator proteins and the formation of long-distance interactions. Blue stars represent currently described genetic polymorphisms.

Despite these advances, only a few studies have addressed how genetic risk variants ultimately cause disease. Some genetic variants are likely to contribute to the generation of epigenetic variation (FIG. 2). The analysis of epigenetic variation in key cell types involved in the pathogenesis of inflammatory rheumatic diseases does not necessarily clarify the functional role of genetic variants identified by GWAS, but could provide valuable insights into the biological mechanisms that initiate and perpetuate disease. Similar to how GWAS began in the field of genetic epidemiology, so epigenome-wide association studies were derived from the burgeoning field of epigenetic epidemiology, with both fields aimed at understanding the molecular basis of disease risk. Whereas genetic risk factors are virtually unalterable, epigenetic alterations might be reversed or modified, making them ideal candidates for targeted therapies.

Epigenetic variation has been associated with various inflammatory rheumatic diseases including SLE, RA and dermatomyositis[27,28]. A study published in 2013 identified two differentially methylated regions within the MHC locus by comparing whole blood samples from patients with RA with those from healthy individuals, suggesting that a proportion of the risk conferred by the MHC region in RA is in fact mediated by altered DNA methylation. High-throughput DNA methylation profiling in blood samples from patients with RA, corrected for cell heterogeneity utilizing a previously described algorithm[29], was used to investigate the occurrence of differentially methylated sites (DMS) in relation to genetic susceptibility[30]. In this study, the investigators proposed that DNA methylation increases phenotypic plasticity in response to a changing environment. The analysis of the associations between SNPs and DMS revealed that half of the SNP–DMS pairs displaying statistically significant correlations are spread over a 5 Mb region covering the MHC cluster and harbouring several RA risk loci. Altogether, these results suggest a direct link between disease-susceptibility SNPs with specific DMS present in the MHC cluster, which together confer the risk of developing RA. Two studies[31,32] compared the epigenome of fibroblast-like synoviocytes (FLS) from patients with RA with that of FLS from patients with osteoarthritis (OA) and identified joint-specific methylome signatures that might have local pathological effects. FLS from the hip and knee of patients with RA displayed thousands of differentially methylated CpG motifs when compared with FLS from patients with OA, indicating a disease-specific methylation signature[31]. These findings reiterate the importance of the epigenetic regulation of inflammation in different pathological contexts, and indicate the potential interaction between genetic and environmental factors that can contribute to disease development.

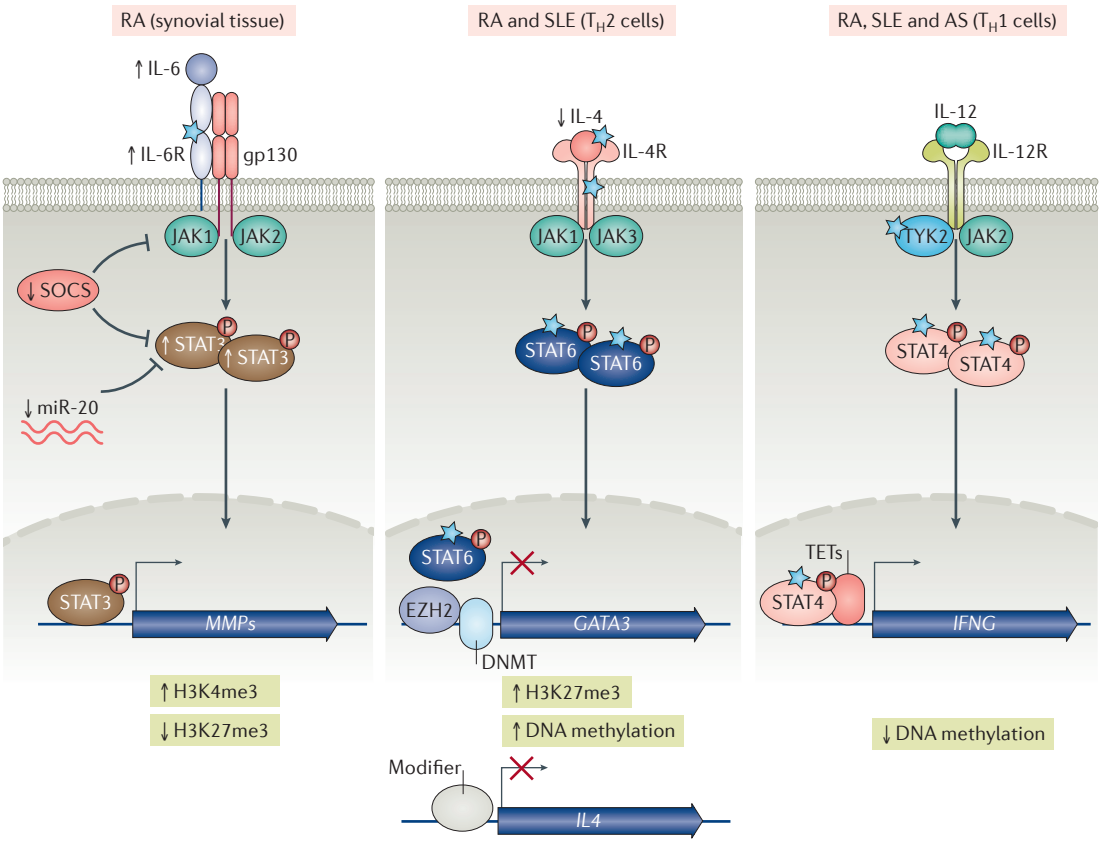
As a disease that is clinically related to RA, juvenile idiopathic arthritis (JIA) is also characterized by a complex interaction between genetic and environmental factors. The methylation profiles of $CD4^+$ T cells correlate not only to the clinical activity of patients with JIA, categorized as active or inactive with flares, but also to their response to anti-TNF therapy[33]. Specifically, the CpG

Table 1 | **Epigenetic alterations in inflammatory rheumatic diseases**

Disease	Cell/tissue type	Epigenetic alteration	Type of alteration	Refs
Rheumatoid arthritis	Fibroblast-like synoviocytes	*LBH* enhancer region hypomethylated	DNA methylation	91
		Enrichment of H3K4me1 at *LBH* enhancer region	Histone modification	91
		Increase in H4ac at *CXCL10* promoter		92
		Increase in H4ac at *IL6* promoter		93
		Downregulation of miR-22	miRNAs	94
		Downregulation of miR-20a		95
	Synovial fibroblasts	Reduced H3K4me3 and increased H3K27me3 in *SFRP1* promoter	Histone modification	96
		Upregulation of miR-203	miRNAs	97
	$CD4^+$ T cells	13 hypermethylated and six hypomethylated CpGs associated with the disease	DNA methylation	98
		Hypermethylation of *FOXP3* upstream enhancer		90
	PBMCs	51,476 DMPs associated with the disease	DNA methylation	30
Systemic lupus erythematosus (SLE)	$CD4^+$ T cells	Hypomethylation of *FOXP3*	DNA methylation	99
		4,839 CpGs correlate negatively with SLE and 1,568 CpGs correlate positively with SLE		39
		1,033 differentially methylated CpGs		79
		Hypermethylation of *IL-2* and *IL-17A* by DNMT3A		100
		Downregulation of miR-26a	miRNAs	39
		Downregulation of miR-142-3p/5p		101
	B cells	166 differentially methylated CpGs	DNA methylation	79
	Monocytes	97 differentially methylated CpGs	DNA methylation	79
		Increase in H3K4me3 at interferon regulatory factor 1 binding sites	Histone modification	102
		Downregulation of miR-302d	miRNAs	103
	PBMCs	Hypomethylation of *IFI44L* promoter	DNA methylation	104
Systemic sclerosis (SSc)	Fibroblasts	Global hypomethylation	DNA methylation	105
		2,710 and 1,021 differentially methylated CpGs in diffuse and limited SSc, respectively		106
		Hypermethylation of *DKK1* and *SFRP1* promoters		106
		Downregulation of miR-193b	miRNAs	107
	$CD4^+$ T cells	Hypomethylation of *CD40LG* in female patients with SSc	DNA methylation	108
	PBMCs	Hypermethylation of *DKK1* and *SFRP1* promoters	DNA methylation	106
Sjögren syndrome	Salivary gland epithelial cells	4,662 differentially methylated CpGs	DNA methylation	109
		Hypomethylation of long interspersed nuclear element 1 (LINE-1)		110
		Degree of hypomethylation correlates with degree of T cell infiltration		111
		Upregulation of miR-768-3p	miRNAs	112
		Downregulation of miR-574		112
	$CD4^+$ T cells	553 hypomethylated and 200 hypermethylated CpGs	DNA methylation	113
		Hypomethylation of *OAS2*		114
	B cells	Hypomethylation of interferon-regulated genes	DNA methylation	114,115
CAPS	Monocytes	Demethylation of *IL1B* and inflammasome genes upon activation	DNA methylation	9
FMF	PBMCs	Hypermethylation of second exon of *MEFV*	DNA methylation	55
		Upregulation of miR-4520a	miRNAs	116

CAPS, cryopyrin-associated periodic syndromes; DMP, differentially methylated position; DNMT3A, DNA methyltransferase 3A; FMF, familial Mediterranean fever; H3K4me1, monomethylated histone H3 lysine 4; H3K4me3, trimethylated histone H3 lysine 4; H3K27me3, trimethylated histone H3 lysine 27; H4ac, acetylated histone H4; miR, microRNA; PBMCs, peripheral blood mononuclear cells.

a Genetically complex inflammatory diseases



b Monogenic inflammatory diseases

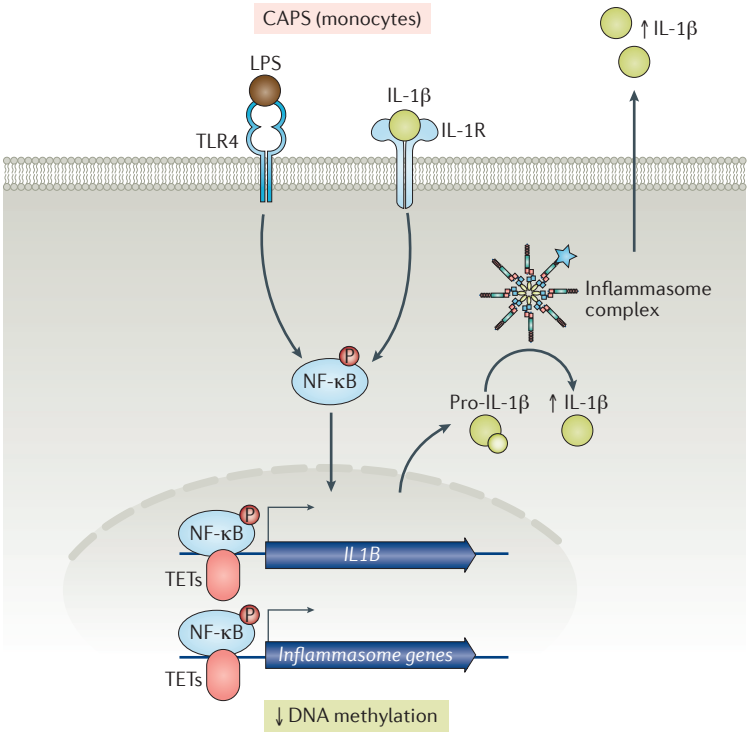


Figure 2 | **Signalling pathways affected by genetic variants and epigenetic alterations in inflammatory rheumatic diseases. a** | In autoimmune diseases, several signalling pathways can be dysregulated by an interplay between specific genetic susceptibility variants and epigenetic dysregulation. Such signalling pathways include the IL-6–signal transducer and activator of transcription (STAT) 3 signalling pathway in synovial cells of patients with rheumatoid arthritis (RA), the IL-4–STAT6 signalling pathway in T helper 2 (T_H2) cells of patients with RA or systemic lupus erythematosus (SLE), and the IL-12–STAT4 signalling pathway in patients with SLE, RA or ankylosing spondylitis (AS). These three examples exemplify Janus kinase (JAK)–STAT signalling involving a cytokine receptor, a JAK kinase and a transcription factor of the STAT family that translocates to the nucleus upon phosphorylation. Blue stars represent currently described genetic polymorphisms. **b** | In monogenic autoinflammatory diseases, the activation of *IL1B* and inflammasome-related genes depends on transcription factor NF-κB, which is activated upon Toll-like receptor (TLR) or IL-1 receptor (IL-1R) stimulation, and epigenetic enzymes such as ten-eleven translocation enzymes (TETs). These mechanisms contribute to the aberrant inflammatory response of patients with cryopyrin-associated periodic syndromes (CAPS) who have mutations in the gene encoding cryopyrin. DNMT, DNA methyltransferases; EZH2, istone-lysine *N*-methyltransferase EZH2; gp130, membrane glycoprotein 130 (also known as IL-6 receptor subunit β); H3K4me3, trimethylated histone H3 lysine 4; H3K27me3, trimethylated histone H3 lysine 27; IL-4R, IL-4 receptor; IL-6R, IL-6 receptor; IL-12R, IL-12 receptor; LPS, lipopolysaccharide; miR-20, microRNA 20; MMPs, matrix metalloproteinases; SOCS, suppressor of cytokine signalling; TYK2, non-receptor tyrosine-protein kinase TYK2.

modules identified as being differentially methylated that correlate with the clinical parameters mentioned above were either located in the MHC locus or were involved in T cell activation. Furthermore, a subset of pathological $CD4^+$ T cells that were aberrantly activated in patients with active JIA was identified by analysing distinct DNA methylation profiles. This finding is consistent with a previous report showing hypomethylation of the 5′ region of *IL32* in $CD4^+$ and $CD8^+$ T cells from patients with JIA[34], which is associated with SNPs identified in the *IL32* gene. Given the role of IL-32 in TNF expression[35] and RA pathogenesis[36], the methylation status of the gene encoding this cytokine might represent a valuable disease biomarker.

Several studies have also identified epigenetic alterations in cell types involved in SLE pathogenesis. Interferon-related genes in neutrophils from patients with SLE have substantial hypomethylation[37], which is consistent with previous findings demonstrating the existence of an interferon signature in peripheral blood mononuclear cells from patients with SLE[38]. Further epigenetic studies have dissected the methylation signatures that correlate with disease activity. As disease activity increases, the epigenetic signature of naive $CD4^+$ T cells undergoes a global shift reflecting T cell activation. This shift consists of hypomethylation of genes involved in T cell activation, including *IL4* and *IL13*, and hypermethylation of genes involved in T cell differentiation and inhibition, such as *TGFB2* (REF. 39). In another study, massive parallel genomic and methylomic sequencing was performed to analyse plasma DNA from patients with SLE who had varying levels of disease activity[40]. Plasma DNA from patients with active SLE showed increased hypomethylation compared with healthy individuals and patients with inactive SLE, which, in agreement with previous reports, might in part be due to DNA shortening[41]. Altogether, epigenetic signatures of SLE activity can be useful as biomarkers to characterize clinical and biological features of this autoimmune disease.

GWAS of patients with ankylosing spondylitis (AS), a debilitating form of chronic autoimmune spondyloarthropathy characterized by axial arthritis, have linked disease susceptibility to the HLA loci, particularly HLA-B27 and its related subtypes[42,43]. Furthermore, combined cross-disease genotyping of several sero-negative inflammatory diseases[23], including AS, revealed several shared variants, such as the missense variant rs2236379 previously associated with Crohn's disease. This variant is located in the *PRKCQ* gene encoding protein kinase Cθ, which is essential for the activation of nuclear factor-κB (NF-κB) and transcription factor AP-1 (REF. 44). Furthermore, the methylation signature of *PRKCQ* was found to be associated with RA susceptibility[30], which might hint at a similar role in AS that could explain the links between the rs2236379 variant and disease susceptibility. A separate study identified rs11209032, a SNP located within the intergenic region between *IL23R* and *IL12RB2*, as associated with disease susceptibility[45]. The authors of this study suggested that this SNP confers a T helper 1 cell signature by regulating the activity of distal enhancers. Epigenetic machineries not only tightly regulate enhancer activity but also mediate long-range interactions between enhancers and promoters[46], highlighting a particular need to dissect genetic-epigenetic interactions to understand their contribution to the development of AS and other autoimmune diseases.

Epigenetic alterations in monogenic inflammatory diseases. Monogenic inflammatory diseases are a group of rare disorders caused by mutations in inflammation-related genes; the best-known examples are FMF and CAPS, which share common features such as recurrent fevers, a prevalence of hyper-reactive innate immune cells and signs of systemic or organ-specific inflammation in the absence of pathogenic infection or autoimmunity. Despite the differences between these syndromes and genetically complex diseases, many of these syndromes fall within the spectrum of autoimmune and autoinflammatory disorders, in which immune and inflammatory cell populations are dysregulated to different extents depending on the patient. In fact, a wide range of clinical manifestations exist among family members with monogenic autoinflammatory diseases who share the same underlying mutation, and some individuals might even display related symptoms in the absence of genetic mutations, suggesting the participation of additional regulatory mechanisms, such as epigenetic dysregulation, in the aetiology of these diseases. By contrast, mutations in monogenic disorders can also have an indirect effect in the dysregulation of epigenetic control (FIG. 2).

FMF is a representative example of an inherited autosomal recessive autoinflammatory disorder characterized by unexplained recurrent fevers and local inflammation in the joints, peritoneum and skin. The hallmark of this disorder is mutations in both alleles of the Mediterranean Fever (*MEFV*) gene, which encodes

pyrin, a pattern recognition receptor expressed on the surface of innate immune cells[47,48]. Pyrin interacts with apoptosis-associated speck-like protein containing a CARD (ASC) and induces the activation of caspase 1 and subsequent activation and release of IL-1β (REFS 49,50). Although all patients with FMF carry mutations in *MEFV*, the disease phenotypes of FMF differ between patients and fall within a spectrum of symptom severity. Although some genotypes correlate with the severity of certain clinical manifestations, such as the Met694Val pyrin variant, which is associated with the degree of subclinical inflammation between fever attacks[51], other genetic variants show almost no correlation to disease activity[52], which is highlighted by the fact that healthy individuals can also harbour *MEFV* mutations[53]. A study of monozygotic twins carrying homozygous mutations in *MEFV* showed variable intra-pair concordance for disease phenotypes, in which some twin pairs displayed identical phenotypes whereas other twin pairs showed variability in disease symptoms, with environmental factors suggested to contribute to approximately 12% of disease phenotypes[54]. Furthermore, a slight but statistically significant hypermethylation signature in the CpG-rich second exon of *MEFV* was detected in leukocytes from patients with FMF, as compared with those from healthy individuals; this signature correlated with a decreased expression of *MEFV*[55].

CAPS are a spectrum of autosomal dominant autoinflammatory diseases that include three distinct phenotypes: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome and neonatal onset multisystem inflammatory disease (NOMID, also known as chronic infantile neurologic cutaneous articular syndrome). In these syndromes, gain-of-function mutations in *NLRP3* are the molecular basis underlying a spectrum of related clinical manifestations[56,57]. The NLRP3 inflammasome is critical for the function of the innate immune system. During an active infection, pattern recognition receptors, including NLRP3, are activated by the binding of ligands such as LPS to TLR4 and initiate downstream signalling cascades[58,59]. The assembly of inflammasome components such as NOD-like receptors and AIM2-like receptors catalyses the proteolytic cleavage and activation of caspase 1, which subsequently cleaves the inhibitory domains of IL-1β and IL-18, converting them into their active forms[60–62]. Both IL-1β and IL-18 are potent inducers of the immune response; IL-1β mediates the recruitment of innate immune cells to the site of infection and IL-18 promotes the production of IFNγ by natural killer cells and cytotoxic T cells[63,64]. Interestingly, no causal relationships have been observed between the location of *NLRP3* mutations and the severity of disease phenotypes in CAPS[65], suggesting a role for additional mechanisms.

One of the first examples of epigenetic alterations in monogenic inflammatory diseases was found in patients with NOMID. Differential expression of several genes encoding histone modifiers and DNA demethylases such as TET2 was observed by comparing lesional skin with non-lesional skin from patients with NOMID[66]. Furthermore, expression of several microRNAs was altered in lesional skin, including the statistically significant downregulation of miR-29c and miR-103-2 (REF. 66). A study published in 2017 investigated the epigenetic dysregulation that might affect the clinical manifestation and response to treatment in patients with CAPS[9]. DNA methylation patterns of monocytes isolated from patients with CAPS (untreated or treated with anti-IL-1 therapy) were analysed before and after stimulation with IL-1β and then compared with those of monocytes from healthy individuals[9]. In healthy monocytes, TET2-dependent demethylation of inflammasome genes, including *IL1B*, *ILR1N*, *NLRC5*, *AIM2*, *PYCARD* and *CASP1* occurred within 24 hours of stimulation with IL-1β. A comparison of unstimulated monocytes isolated from patients with CAPS with those from healthy individuals revealed an almost identical methylation pattern of inflammasome genes. However, in IL-1β-stimulated monocytes from patients who were not receiving anti-IL-1 treatment, the demethylation of inflammasome genes was more efficient than that of monocytes from patients receiving anti-IL-1 therapy and healthy individuals. These findings demonstrate the presence of an altered epigenetic control of inflammasome genes in monocytes from patients with CAPS, which triggers a rapid activation of gene expression upon IL-1β stimulation. Interestingly, each patient with CAPS harboured distinct mutations in the *NLRP3* gene, again supporting the notion that the genetic mutations alone might not confer specific clinical or molecular phenotypes. Moreover, epigenetic alterations of inflammasome genes seem to be independent from the type of mutation[9].

Overall, research on the spectrum of monogenic autoinflammatory diseases, although well-characterized in their genotypes, does not completely explain the wide range of clinical manifestations. In the past 5 years, researchers have only begun to scrape the tip of the iceberg with regard to epigenetic alterations that correlate to disease phenotypes. The investigation of epigenetic defects will not only enrich our understanding of the biological mechanisms behind these devastating diseases, but might also provide useful and novel tools for clinical interventions.

Therapeutic targets

Over the past 10 years, advances in the understanding of how epigenetic mechanisms confer specific disease phenotypes have not only enabled the improvement of current therapeutic strategies, but have also provided a path to the discovery of novel therapeutic targets. Pharmacological treatments can target epigenetic mechanisms in two ways; by directly modifying enzymes that catalyse epigenetic changes, or by targeting factors that indirectly affect global epigenetic profiles. For genetically complex inflammatory diseases, epigenetic alterations are particularly relevant. To date, histone deacetylase (HDAC) inhibitors have been extensively studied as potential therapeutic agents because the expression of HDACs is increased in several inflammatory diseases[67,68] (TABLE 2). The expression levels of class I HDACs in synovial tissue from patients with RA

correlates with increased levels of inflammatory mediators[69]. Furthermore, global acetylation of histone H3 and histone H4 is decreased in $CD4^+$ T cells from patients with active SLE compared with $CD4^+$ T cells from patients with inactive SLE and healthy individuals, indicating that histone H3 acetylation inversely correlates with disease severity[70]. This finding also suggests that increased levels of HDACs might contribute to SLE pathogenesis. Further studies have demonstrated that HDAC inhibitors are potent anti-inflammatory agents, as they suppress LPS-induced cytokine production[71]. The HDAC inhibitor ITF2357, which targets class I and class II HDACs, is able to ameliorate inflammation and prevent joint destruction in experimental arthritis[72,73]. In a phase II clinical trial, 17 patients with systemic JIA treated with ITF2347 for 12 weeks showed substantial improvements in disease, as demonstrated by a reduction in the number of joints with active disease or with limited range of motion, compared with before treatment[74] (TABLE 2). Although no HDAC inhibitors have been tested in clinical trials for the treatment of RA and SLE, several *in vitro* studies have shown that these agents are effective in the suppression of disease phenotypes[67,75]. For instance, the oral HDAC inhibitor MPT0G009 inhibits cytokine release in activated macrophages from patients with RA and reduces osteoclast formation by inhibiting the activity of the trancription factors NF-κB and NFATc1 (REF. 76). Another study showed that the HDAC inhibitor trichostatin A is able to reduce the transcription of *IRF5* by inhibiting the activity of its promoter; given the relevance of increased *IRF5* expression in childhood-onset SLE, these data might provide a mechanistic rationale for the use of HDAC inhibitors for the treatment of SLE[77].

FLS and synovial fibroblasts play important roles in the pathogenesis of local inflammation in the joints of patients with RA, and both show altered global methylation patterns. Global hypomethylation, which correlates to decreased levels of *DNMT1* expression, was detected in synovial fibroblasts from patients with RA as compared with those from patients with OA[78]. Furthermore, promoter hypomethylation is often observed in T cells from patients with SLE[39], particularly in the genes encoding IFNα (REF. 79), suggesting that these cells have a high baseline expression level of IFNα. Interestingly, the treatment of synovial fibroblasts[78] and $CD4^+$ T cells[80] from healthy individuals with the FDA-approved DNMT inhibitor 5-azacytidine conferred gene expression profiles that resemble cells from RA and SLE disease models, respectively. Therefore, the use the DNMT inhibitors is not a feasible option for the treatment of these diseases. Conversely, targeting factors that promote the upregulation of DNMT expression could be an effective strategy. One such example is PP2Aα, which inhibits the expression and activity of DNMT1 in T cells through the inhibition of the MAPK and ERK (MEK)–ERK signalling pathway[81]. Alterations in this signalling pathway caused by defective activation of protein kinase Cδ induces lupus-like autoimmunity in mice[82]. Given that PP2Aα expression is increased in patients with SLE or FMF compared with healthy individuals[83], these findings could provide a mechanistic

Table 2 | **Therapeutic treatments targeting epigenetic alterations in inflammatory rheumatic diseases**

Compound	Clinical effects	Epigenetic mechanisms	Disease	Phase	Refs
IT2357 (Givinostat)	Anti-inflammatory with reduction in pain and active disease in joints	Inhibitor of class I and class II HDACs	sJIA	Phase II clinical trial	74
Trichostatin A	Anti-fibrotic	Inhibitor of class I and class II HDACs	SLE	Pre-clinical	77,117
			SSc		118
MPT0G009	Anti-inflammatory with inhibition of bone destruction	Inhibitor of class I and class IIb HDACs	RA	Pre-clinical	76
SAHA/ Vorinostat	Anti-fibrotic	Inhibitor of HDACs	RA	Pre-clinical	76
Methotrexate	Anti-inflammatory with reduction of pain and joint inflammation as well as prevention of disease progression	Reversal of DNA hypomethylation in PBMCs and restoration of regulatory T cell function through demethylation of *FOXP3*	RA	FDA-approved	119,90
Tocilizumab	Anti-IL-6 receptor antibody that slows down disease progression	Downregulation of IL-6 in B cells by induction of DNA hypermethylation	SLE	FDA-approved for RA and sJIA	120
Anakinra/ Canakinumab	Reduction in disease severity, including reversal of inflammation-mediated loss of organ function	Reversal of rapid demethylation of inflammasome genes upon activation of monocytes	CAPS	FDA-approved	9

CAPS, cryopyrin-associated periodic syndromes; HDAC, histone deacetylase; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SAHA, suberoylanilide hydroxamic acid; sJIA, systemic juvenile idiopathic arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Box 2 | **Epigenomic analysis in the context of clinical studies**

The availability of low-cost methods for epigenomic analyses, together with the development of bioinformatic tools, has contributed to the growth of studies aimed at profiling DNA methylation and histone modifications in immune cells isolated from patients with inflammatory rheumatic diseases. When studying DNA modifications in large cohorts of patients, bead arrays are recommended as they ensure a coverage of ~1 million CpG sites, in combination with bisulfite modification and oxidative bisulfite modification to obtain both 5-methylcytosine and 5-hydroxymethylcytosine profiles. However, for a wider coverage, whole genome bisulfite sequencing is recommended, although the cost of this technique is generally too high for the analysis of large cohorts of patients. Chromatin immunoprecipitation combined with deep sequencing (ChIP-seq) is a robust tool to investigate histone modification profiles, as well as the distribution of transcription factors or epigenetic enzymes. However, for these experiments, samples from patients require adequate preparation, including formaldehyde crosslinking, before storage.

explanation for the global hypomethylation observed in these patients, as well as a potential target for clinical intervention.

Epigenomics in personalized medicine

Epigenomic profiles are not only useful for understanding the molecular mechanisms underlying disease development, but they also provide insight into clinical features that might predict disease outcome or response to certain therapeutic treatments (BOX 2; TABLE 3). In the past 10 years, the idea of personalized medicine has begun to take shape in the context of cancer treatment[84,85]. Although this approach is still far from being applicable to inflammatory rheumatic diseases, the variable responses of patients to currently available therapies highlight the need of more precise therapeutic strategies. Several aspects need to be considered in future studies. First, genetic and epigenetic aberrations need to be identified to tailor treatments to individual patients. The development of tools to integrate genomic and epigenomic data has already enabled the quantification of the epigenetic contribution to genetically complex diseases. By mapping the global methylation status of several cell populations in the blood of patients with RA, namely $CD14^+$ monocytes, $CD4^+$ and $CD8^+$ T cells and $CD19^+$ B cells, with genotype data, a group of researchers identified nine putative DMS that mediate the genetic risk of RA[30]. Another study that employed computational methods on available data to identify associations between SNPs and histone marks, showed that 31 SNPs associated with RA correlated with >1,000 trimethylated histone H3 lysine 4 (H3K4me3) peaks in different tissues, with most associations occurring in regulatory T cells[86]. Advances made in the past 2 years in the consolidation of epigenomic megadata, including DNA methylation and histone modifications, from different cell types and in the context of different autoimmune diseases, such as the development of the International Human Epigenome Consortium Data Portal[87], will enable the direct comparison between individual genetic and epigenetic profiles and specific reference human epigenomes. This comparison, which is both disease-specific and tissue-specific, will guide personalized treatment and disease management.

Second, it is of great interest to identify epigenetic biomarkers that will aid the early detection of disease, prognosis and response to therapy (TABLE 3). A correlation between specific epigenetic signatures and disease severity has been described for various inflammatory rheumatic diseases. For example, a positive correlation between hypomethylation of plasma DNA and levels of circulating anti-double stranded (ds) DNA antibodies was found in plasma from patients with active SLE[88]. Given that the presence of anti-dsDNA antibodies has been associated with the presence of a high number of risk alleles in patients with SLE[88], the methylation signature of plasma DNA can be a potential indicator of disease outcome in SLE. Furthermore, a study published in 2016 (REF. 33) identified specific methylation profiles in $CD4^+$ T cells from patients with JIA, which correlated with disease activity and response to anti-TNF therapy. Finally, a study found that four CpGs were differentially methylated in T cells from patients with RA who were non-responders to DMARD therapy, compared with responders[89].

The ultimate aim of precision medicine is to combine treatment with targeting epigenetic regulators. Several currently available therapies alter the epigenetic profiles of patients with inflammatory rheumatic diseases. For example, DMARDs cause the demethylation of *FOXP3*, leading to its increased expression and the subsequent restoration of regulatory T cell function[90]. However, the mechanisms behind the epigenetic effects of these drugs remains unclear. The discovery of specific synthetic drugs targeting individual epigenetic aberrations in inflammatory rheumatic diseases is urgently needed. Given the genetic and phenotypic complexity of these diseases, therapies directed against a single target might not benefit all patients. Hence, to achieve optimal treatment efficacy for each patient, the type of medication, dosage and patient response also need to be taken into account in the design of personalized therapies.

Conclusions

To dissect the molecular mechanisms that underlie genetically complex and monogenic inflammatory diseases, the relative contributions of genetic and environmental factors to disease development need to be understood. Although genetic alterations in some of these diseases are well characterized, the complete picture of how epigenetic mechanisms contribute to disease phenotypes remains to be determined. A common feature of inflammatory disorders is the dysregulation of immune cells, at the levels of both differentiation and activation. Given the crucial role of epigenetic mechanisms such as DNA methylation and histone modifications in modulating immune cell identity and function, epigenetic alterations are clearly important contributors to disease development. Several studies have highlighted differences between the methylomes of cells from patients with inflammatory disease and those of cells from healthy individuals, with many of these differences being specific to disease type and severity. The characterization of distinct epigenomic profiles will ultimately give rise to the discovery of relevant epigenetic biomarkers indicative of disease type, prognosis and response to treatment.

Table 3 | **Epigenetic biomarkers in inflammatory rheumatic diseases**

Disease	Samples	Epigenetic alteration	Comments	Refs
Rheumatoid arthritis	Whole blood	Differentially methylated CpGs in the MHC region	90 DMSs are genotype-dependent; 9 DMSs mediate genetic risk in RA	30
	Whole blood	Two differentially methylated positions in exon 7 of *LRPAP1*	Hypermethylation is associated with non-responsiveness to etanercept	121
	T cells	21 differentially methylated CpGs	Differential methylation between responders and non-responders to DMARD treatment	89
sJIA	$CD4^+$ T cells	Differentially methylated CpGs in the MHC locus	Differentially methylated CpGs are associated with clinical activity and display a T cell activation signature	33
SLE	Plasma DNA	Hypomethylation of plasma DNA	Hypomethylation of plasma DNA correlates positively with a high level of circulating anti-double stranded DNA antibodies, which is associated with poor disease outcome	88

DMS, differentially methylated site; sJIA, systemic juvenile idiopathic arthritis; SLE, systemic lupus erythematosus.

Furthermore, unlike genetic mutations, epigenetic modifications are inherently reversible, making them attractive therapeutic targets. Since 2006, several drugs targeting chromatin modifiers, including inhibitors of DNMTs and HDACs, have been approved for the treatment of haematological malignancies. These advances provide hope for discovering treatments that target specific mechanisms of inflammatory rheumatic diseases. The challenge now is to understand how epigenetic alterations affect different cell types involved in pathogenesis using next-generation, high-throughput technologies, and to discover new ways to translate this information into a clinical setting.

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The case for periodontitis in the pathogenesis of rheumatoid arthritis

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Abstract | Rheumatoid arthritis (RA), an autoimmune disease that affects ~1% of the human population, is driven by autoantibodies that target modified self-epitopes, whereas ~11% of the global adult population are affected by severe chronic periodontitis, a disease in which the commensal microflora on the tooth surface is replaced by a dysbiotic consortium of bacteria that promote the chronic inflammatory destruction of periodontal tissue. Despite differences in aetiology, RA and periodontitis are similar in terms of pathogenesis; both diseases involve chronic inflammation fuelled by pro-inflammatory cytokines, connective tissue breakdown and bone erosion. The two diseases also share risk factors such as smoking and ageing, and have strong epidemiological, serological and clinical associations. In light of the ground-breaking discovery that *Porphyromonas gingivalis*, a pivotal periodontal pathogen, is the only human pathogen known to express peptidylarginine deiminase, an enzyme that generates citrullinated epitopes that are recognized by anti-citrullinated protein antibodies, a new paradigm is emerging. In this Review, the clinical and experimental evidence supporting this paradigm is discussed and the potential mechanisms involved in linking periodontitis to RA are presented.

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The economic and social burden of dental disease is extremely high, with direct treatment costs estimated at 416 billion US dollars per year worldwide, which corresponds to an average of 5.8% of global health expenditure[1]. The majority of this spending (~75%) goes towards treating periodontitis, an inflammatory disease affecting the tissues that support the teeth, including the alveolar bone. Arguably, periodontitis is the most prevalent bacteria-driven chronic inflammatory disease in humans; severe forms of periodontitis affect ~11% of the global adult population, with the prevalence being equally high (7.2–9.8%) in most developed countries[2]. In the USA alone, 64.7 million people (46% of adults) have periodontitis, with 8.9% of these individuals having severe disease[3]. In addition, mounting evidence suggests a strong link between periodontitis and systemic diseases such as rheumatoid arthritis (RA).

Despite having differing aetiologies, periodontitis and RA share many features. Both are multifactorial diseases characterized by localized chronic inflammatory reactions that are fuelled by a similar set of cytokines (TNF, IL-6 and IL-17)[4,5]. Both diseases also result in high concentrations of inflammatory markers such as C-reactive protein (CRP) in the circulation[6,7]. Furthermore, bone erosion driven by pro-inflammatory T helper 17 (T_H17) cells is a pathological hallmark of both diseases[8]. Finally, periodontitis and RA also share environmental and genetic risk factors.

Until a few years ago, these common features made it difficult, if not impossible, to unequivocally answer questions about a potential causative link between the two diseases. The discovery and characterization of an enzyme expressed uniquely by the primary periodontal pathogen *Porphyromonas gingivalis*, peptidylarginine deiminase (known as PPAD to distinguish this bacterial enzyme from human peptidylarginine deiminases)[9], formed the basis of the hypothesis that PPAD-mediated protein citrullination at inflamed periodontal sites can initiate a cascade of events that culminate in the production of anti-citrullinated protein antibodies (ACPAs) and, eventually, in the clinical manifestation of RA[10]. In this Review, we critically discuss the results of epidemiological, serological, clinical and experimental studies that explore the links between periodontitis and RA. We will focus particularly on citrullinated epitopes, the breakdown of immune tolerance and the generation of ACPAs specific for proteins present in chronically inflamed periodontal tissue.

Aetiology of periodontitis

Periodontitis results from dysbiosis of the oral microbiota[11] (BOX 1). Pathological changes in the periodontium (FIG. 1) are strongly associated with a small group of anaerobic, Gram-negative bacteria, referred to as the 'red complex', which includes *P. gingivalis*, *Treponema*

Key points

- Periodontitis and rheumatoid arthritis (RA) are closely linked, and periodontitis often precedes the development of RA
- Periodontitis correlates with levels of anti-citrullinated protein antibodies in healthy individuals, suggesting that periodontitis could trigger the autoimmune response that leads to RA
- Hypercitrullination of proteins at chronically inflamed sites of periodontitis could constitute the mechanistic link between periodontitis and RA
- The major periodontal pathogens *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* are directly implicated in the breakdown of immune tolerance to citrullinated epitopes
- Further well-designed mechanistic and epidemiological studies into the links between periodontitis and RA are needed to elucidate the mechanisms involved

denticola and *Tannerella forsythia*. The bacteria of the red complex, together with *Aggregatibacter actinomycetemcomitans*, are unwelcome visitors in the diverse microbiome present on the tooth surface. Once stable colonization of the gingival sulcus, the natural gap between the tooth and the surrounding gum tissue, is achieved, these bacteria disrupt the healthy composition of the community of commensal bacteria in a process known as microbiota shift. Together with the proliferation of red complex species on the tooth surface below the gum line, colonization triggers a response by the host's innate immune system. Once entrenched in the dysbiotic biofilm, the pathogens are resistant to attack by phagocytes and bactericidal proteins, peptides and reactive oxygen species, and are thus difficult to eradicate, leading to the development of a chronic inflammatory reaction[12]. Periodontal tissue damage and the erosion of tooth-supporting structures (FIG. 1) are therefore likely to be caused by a deregulated innate immune response against the bacteria rather than by the bacteria themselves. Despite the appearance of an ongoing localized 'battle', the consequences of inflammation spill out of the periodontal pockets in the form of overactive neutrophils and high levels of inflammatory molecules (such as cytokines and CRP), which can be detected in the blood of patients with periodontitis[13,14].

Aetiology of RA

RA, a systemic autoimmune disease characterized by chronic, painful inflammation of the joints, disability and increased mortality, affects 0.5–1.0% of the global population[15]. The cause of RA is still unclear; however, RA is thought to be triggered by a combination of genetic and environmental factors that lead to the breakdown of immune tolerance at mucosal surfaces, specifically the lungs, gut and periodontium[16,17]. The ensuing autoimmune response is characterized by the production of rheumatoid factor and ACPAs[18]. The binding of ACPAs to citrullinated epitopes in the joints and the formation of immune complexes containing rheumatoid factor fuel a vicious cycle of tissue damage involving the activation of synovial macrophages and dendritic cells, and the release of pro-inflammatory cytokines and tissue-degrading enzymes[19]. Simultaneously, peptidylarginine deiminases (PADs) released from neutrophils during necrosis or during the production of neutrophil extracellular traps (NETs; a process known as NETosis), citrullinate proteins in the joints, resulting in a sustained, self-sustaining local immune reaction[20]. The aberrant response to citrullinated epitopes seen in patients with RA is strongly associated with a particular set of polymorphisms of *HLA-DRB1*, referred to as the 'shared epitope' and, to a much lesser extent, with polymorphisms in >100 different loci that are (mostly) implicated in immune pathways[21].

Post-translational protein modification

The diagnosis of RA was revolutionized by the discovery of ACPAs. These antibodies occur in ~70% of patients with RA and are; disease-specific; strongly associated with major genetic and environmental risk factors; associated with severe disease, suggesting pathogenic involvement; and are detectable in the circulation <10 years before any clinical symptoms of RA are seen[22], indicating that the initial loss of immune tolerance towards citrullinated proteins is likely to be the consequence of an inflammatory event occurring outside the joint. However, the majority of proteins in our body undergo some form of post-translational modification, ranging from proteolytic processing, glycosylation or lipidation, to the enzymatic or chemical modification of selected residues. These protein modifications are essential physiological processes that are required for the normal function of our bodies; nevertheless, by generating neo-epitopes, such modifications can lead to the generation of anti-modified protein antibodies (AMPAs), such as ACPAs, in genetically susceptible individuals who are exposed to specific environmental risk factors. Interestingly, in addition to ACPAs, several AMPAs are associated with RA, including autoantibodies that react with the hinge region of fragmented IgG and autoantibodies recognizing acetylated, carbamylated and malondialdehyde-acetaldehyde-modified lysine residues in polypeptide chains[23]. Despite these new discoveries, the following section focuses exclusively on citrullination and ACPAs, as these are the only RA-associated autoantibodies to have been investigated in the context of periodontitis.

Citrullinated proteins

The post-translational modification of proteins by the enzymatic deimination of arginine residues converts this positively charged residue into a neutral citrulline residue. Citrullination occurs naturally *in vivo*, and has an important role in the normal functioning of the immune system and in physiological processes such as skin keratinization, the insulation of neuronal axons, maintaining plasticity in the central nervous system and in gene regulation via chromatin remodelling[24]. Additionally, deimination of arginine residues can occur under pathologic inflammatory conditions associated with apoptosis and necrosis of neutrophils, as well as during NETosis, when the hypercitrullination of histones is critical for the generation of NETs as part of the innate response to bacterial infection[25]. So, in addition to RA, the citrullination of proteins

Box 1 | **Changing views on the pathobiology of chronic periodontitis**

Periodontal disease (periodontitis) has always been recognized as an inflammatory disease, but our understanding of the underlying pathobiology is constantly evolving[108]. Between the 1950s and the early 1970s, the accumulation of nonspecific bacterial plaque on the tooth surface below the gum line was generally believed to initiate and drive the development of disease[109]. This perception changed when researchers noticed that not all the organisms present in dental plaque are equipped with virulence factors capable of causing destructive periodontitis[110]. Advances in the cultivation and identification of microorganisms detected in periodontal pockets lead to the recognition of a group of predominantly Gram-negative, anaerobic bacteria that were strongly associated with periodontitis[111]. Based on these results, the specific plaque hypothesis was formulated in the late 1970s[112,113]. According to the emerging dogma, bacterial species such as *Actinobacillus* (now *Aggregatibacter*) *actinomycetemcomitans*, *Bacteroides* (now *Porphyromonas*) *gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* (now *Tannerella forsythia*), *Campylobacter rectus*, *Eubacterium nodatum*, *Fusobacterium nucleatum*, *Streptococcus intermedius* and *Treponema* spp. were implicated as periodontal pathogens. The three most frequently encountered bacterial species in the disease sites — *P. gingivalis*, *T. forsythia* and *Treponema denticola* — were referred to as the 'red complex' (REF. 114).

Simultaneously, data from co-aggregation studies of dental plaque bacteria lead to the concept of a gradual, temporal formation of a biofilm, starting with early colonizers and moving towards late colonizers, with the latter composed of abundant red complex pathogens[115]. Changes in the composition of the microflora over time corresponded to changes in the health status of the adjacent gingival tissues[116]. Based on these findings, a hypothesis of reciprocal interactions between the bacterial community and the host tissues was formulated in 2005 (REF. 116). Over the past 10 years, advances in high-throughput techniques for identifying bacteria lead to the revelation that the tooth surface is home to >600 microbial taxa; enabled the identification of new potential pathogens (such as *Filofactor alocis*); and led to the confirmation of the association between *P. gingivalis* and periodontitis[117]. In 2011, *P. gingivalis* was recognized as being a keystone pathogen responsible for elevating the virulence of the entire microbial community, even if the organism itself was only present in low numbers[118]. In this new model, the pathogenesis of periodontitis is explained by synergy between *P. gingivalis* and other putative keystone periodontal pathogens initiating dysbiosis, and the ensuing inflammation being exacerbated by the overgrowth of inflammation-loving potential pathogens[119].

and peptides has been linked to primary open-angle glaucoma, nephropathy, multiple sclerosis, Alzheimer disease and psoriasis[25].

Protein citrulination by PADs. Protein citrullination is catalysed by endogenous PADs (FIG. 2), which are thought to form the 'cornerstone' of RA pathogenesis. Five PADs have been characterized in humans and other mammals, each of which has a different tissue distribution[26]. PAD1 and PAD3 are found in the epidermis and in hair follicles and are principally cytoplasmic enzymes. PAD2 is expressed in a variety of tissues throughout the body, including muscle, brain and haematopoietic cells. PAD4 (formerly known as PAD5) is found primarily in haematopoietic cells and is the only member of the family that resides within the nucleus. Finally, PAD6 is expressed in ova, early embryos and the thymus[26].

PAD2 and PAD4 require relatively high calcium concentrations (>5 mM) to be active *in vitro*[27]; such concentrations exceed those found in plasma and synovial fluid by ~3–5 times[28]. Discrepancies between *in vitro* and *in vivo* calcium requirements imply the existence of as yet undiscovered factors that are capable of modulating the sensitivity of PADs to calcium ions, the deregulation of which might underpin pathological citrullination, increased levels of ACPAs and, ultimately, the development of RA. Indeed, a small subset of anti-PAD4 autoantibodies associated with erosive RA had a substantial stimulatory effect on PAD4 activity[29]. Molecular modelling suggests that these antibodies bind to PAD4 and lock its structure into a 'fully active' conformation that is characteristic of the calcium-saturated enzyme. Of note, although PAD4 is a specific autoantigen in patients with RA, their reaction to it is polyclonal, and anti-PAD4 antibodies purified from the sera of patients with RA can also inhibit citrullination of fibrinogen by the enzyme *in vitro*[30]. Such autoantibodies clearly have different effects on PAD4 activity depending on which epitopes they bind to.

Citrullinated proteins in RA. Citrullinated proteins can be found in irritated lungs[31], in the inflamed periodontium of patients with periodontitis[32] and in the gut[33]; the level of protein citrullination is increased in the intestines of patients with RA compared with healthy individuals and citrullinated peptides derived from 121 different proteins, including peptides associated with RA, have been identified there[33]. Interestingly, hypercitrullination in the gums is associated with the presence of *A. actinomycetemcomitans* in the periodontal pockets[34]. Similarly, citrullination in the lungs is associated with smoking[31], thereby mechanistically linking this environmental risk factor with ACPA production. These findings not only confirm that citrullination occurs at disparate sites in the human body but also support the theory that pathogenic autoimmune reactions might be initiated at sites other than the joints. The gut mucosa, with a vast array of microbiota that are prone to dysbiotic changes, could be such a site, as suggested by the results of several studies using clinical samples and animal models[35–37]. Treating RA resolves dysbiosis of microbiota in the gut and mouth, further supporting this theory[38].

The theory of a causal link between abnormal citrullination, loss of immune tolerance, ACPAs and RA was strengthened by data from genome-wide association studies showing the presence of *PADI* family genes, which encode PADs, within the RA susceptibility locus. A case–control linkage disequilibrium study based on single-nucleotide polymorphisms (SNPs) revealed that the RA susceptibility haplotype in *PADI4* (but not in the neighbouring *PADI* genes) resulted in the expression of a more stable transcript than that produced by the gene without the SNP, and that this SNP is strongly associated with high levels of ACPAs in the serum of individuals with RA[39]. Taken together, these findings indicate a pivotal role for PADs in the pathogenesis of RA, in which increased citrullination of proteins in a cytokine-rich, inflammatory milieu eventually leads to the breakdown of tolerance to citrullinated epitopes presented by specific HLAs.

Links between RA and periodontitis

As a chronic inflammatory disease, some of the characteristics and pathogenic processes of RA mirror those of periodontitis, with both diseases ultimately resulting in the progressive destruction of bone. The deep

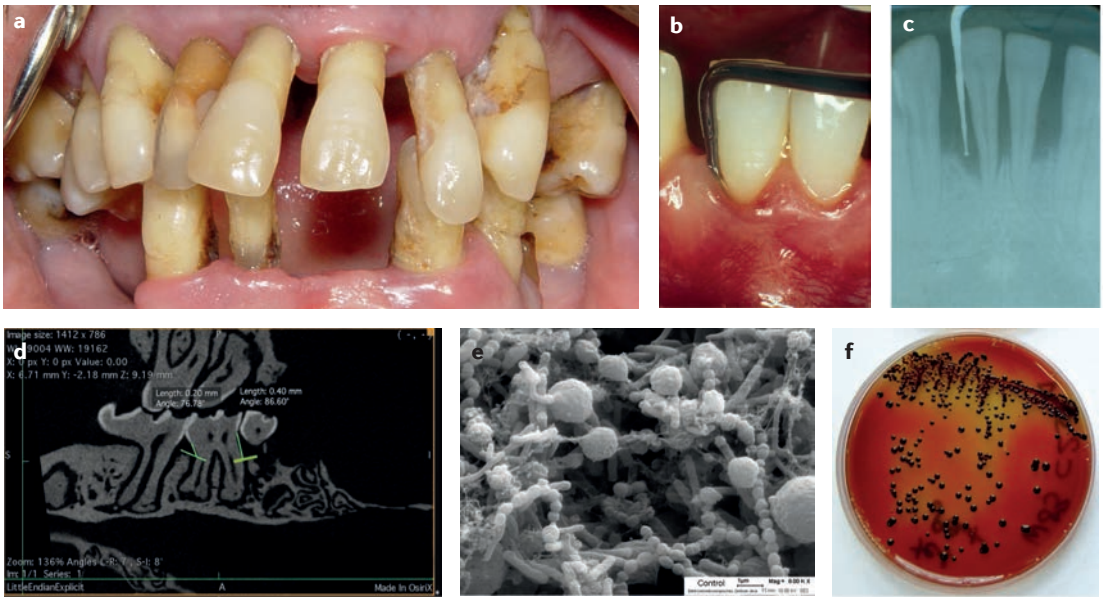


Figure 1 | **Clinical and microbiological aspects of periodontitis.** The clinical picture of chronic periodontitis is defined by the formation of periodontal pockets ≥4 mm deep with accompanying alveolar bone loss resulting from chronic inflammation. Subsequent loss of tissue attachment to the teeth and deepening of the periodontal pockets to 10–12 mm creates the perfect bacterial niche for harbouring a polymicrobial community, including the key periodontitis pathogen *Porphyromonas gingivalis*. **a**| Inflammation of the gingiva. **b** and **c**| Measurement of periodontal pocket depth using a periodontal probe. **d**| Advanced levels of bone loss visualized by X-ray. Green lines indicate level of alveolar bone loss. **e**| Scanning electron micrograph showing the complex dysbiotic bacterial biofilm (dental plaque) collected from the tooth surface below the gum line. **f**| Growth of the major periodontal pathogen, *P. gingivalis* on blood agar. Image in part **a** provided by Andreas Stavropoulos, Department of Periodontology, Faculty of Odontology, Malmö University, Malmö, Sweden. Images in parts **b** and **c** provided by Wolfgang Pfister, Institute of Medical Microbiology, University Hospital Jena, Jena, Germany. Image in part **e** provided by Sandor Nietzsche, Centre of Electron Microscopy, University Hospital Jena, Jena, Germany.

inter-relationship between these two diseases is the result of shared genetic and environmental risk factors, including expression of *HLA-DRB1*, smoking and other exogenic risk factors such as nutrition, socioeconomic status and psychological factors (such as stress)[40–42]. Notably, the deleterious effects of smoking are limited to ACPA-positive patients with RA[42]. Despite obvious differences in the aetiology of RA and periodontitis, evidence exists linking the two diseases clinically, epidemiologically, serologically and experimentally[43,44].

Clinical evidence

Several case–control studies have shown that periodontitis is more prevalent in patients with active RA than in healthy individuals; conversely, the prevalence of RA is also higher in individuals with periodontitis than in those without periodontitis (TABLE 1). The largest case–control study conducted to date (which included 287 patients with RA and 330 patients with osteoarthritis acting as controls) revealed an independent relationship between periodontitis and established seropositive RA after accounting for multiple potential confounders, including the presence of the *HLA-DRB1* shared epitope, smoking and oral hygiene[45]. In addition, when compared with the clinical course of periodontitis in individuals who do not have RA, the course of periodontitis in patients with RA was more severe and independent of age, sex, ethnicity or smoking history[45]. By contrast, despite including 6,682 participants, a 2016 case–control study from the Swedish population-based Epidemiological Investigation of Rheumatoid Arthritis (EIRA) cohort linked with the Swedish National Health Registry found no association between RA and periodontitis, or between seropositive RA and periodontitis[46]. Unfortunately, this study had some limitations, including a lack of information regarding comorbidities and other confounding factors, meaning that the question of whether microbial subversion tips the balance from homeostasis to disease in the joints remains unanswered. The reasons for conflicting epidemiological findings might include the nonspecific classification criteria applied to periodontitis, the size of patient cohorts used in these studies and the lack of data regarding confounding factors and treatments (TABLE 1). To better understand the relationship between periodontitis and RA, retrospective studies that lack clinical periodontitis measurements should focus on the presence of *P. gingivalis* infection, which can be estimated objectively by measuring specific antibody titres, rather than using patient surveys to define periodontitis status.

The unique ability of *P. gingivalis* to citrullinate proteins via PPAD provides a potential causal link between periodontal infection and RA. To date, the most robust and reliable data supporting this link are based on the

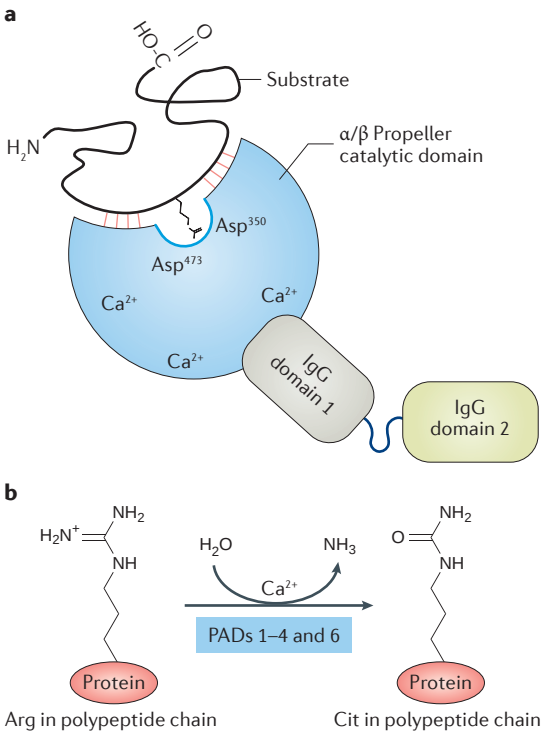


Figure 2 | **Protein citrullination catalysed by human peptidylarginine deiminases. a**| In human peptidylarginine deiminase (PAD)2 and PAD4, a ~375 residue calcium dependent α/β propeller catalytic domain is preceded by two IgG domains. The substrate interacts with the main chain of the reactive site cleft on both sides of the deiminated arginine side chain (indicated by red lines), while the arginine guanidine group interacts with the catalytic residues (Asp_{350} and Asp_{473}). **b**| Human PADs catalyse the hydrolytic, calcium-dependent conversion of arginine residues within a polypeptide chain to citrulline residues, releasing ammonia in the process.

EIRA cohort; researchers examined the serum titre of antibodies against gingipain R (RgpB), a conserved proteolytic enzyme expressed by *P. gingivalis*, in 1,974 patients with RA and 377 healthy individuals[47]. The association between anti-RgpB antibody levels and RA in patients who were ACPA-positive was stronger than that between RA and well-established risk factors such as smoking[47]. This finding is consistent with the results of previous smaller scale studies[48,49]. Similarly, when purified PPAD was used as an antigen, there was a positive correlation between serum levels of ACPAs and anti-PPAD antibodies[50]. Interestingly, patients with RA who were treated with a biologic DMARD showed a significant reduction in anti-PPAD antibody titres after 3 months and 6 months of therapy ($P=0.04$), which correlated with a reduced 28-joint Disease Activity Score (DAS28)[51]. Thus, one of the pivotal factors responsible for the outcome of serological studies linking periodontitis with RA seems to be choosing an antigen that enables measurement of the immune response against *P. gingivalis* with a high-degree of specificity. For example, the EIRA cohort was screened using purified *P. gingivalis*-specific RgpB protein, and the results showed a strong association between anti-RgpB antibody levels and RA[47]. Conversely, a large study that used *P. gingivalis* cell lysates as an antigen failed to detect a statistically significant difference in the titre of anti-*P. gingivalis* antibodies between patients with RA and healthy individuals or a positive correlation between ACPAs and anti-*P. gingivalis* antibodies in patients with RA[45]. This discrepancy might be due to previous host immune responses to other bacterial infections crossreacting with *P. gingivalis* lysates.

The close relationship between periodontitis and RA, particularly the association between the immune response to *P. gingivalis* and ACPA levels in healthy individuals who go on to develop RA at a later date (so-called 'pre-RA') supports an aetiological role for *P. gingivalis* in the pathogenesis of RA[52,53]. This theory is supported by the results of the Nagahama study, which reported a correlation between ACPAs and periodontitis parameters (such as the number of missing teeth, community periodontal index and clinical attachment loss) in 9,554 healthy adults[54]. Taken together, these data strongly support the role of periodontitis in ACPA generation and fit well into the 'two hit' model of pathogenesis[55]. The hypothesis that the breakdown of tolerance is due to PAD-dependent or PPAD-dependent citrullination in periodontal tissues is well supported[10]. Similarly, it is thought that citrullination associated with pathogenic immune reactions in the lungs of cigarette smokers or in a gut with a dysbiotic microbiome might (eventually) lead to a breakdown of immune tolerance and increased ACPA production, which can precede the clinical manifestation of RA by many years[22]. These scenarios most likely represent the 'first hit'; unfortunately, the secondary factor(s) that triggers disease in the joints remains unknown. Such a factor could involve infectious, mechanical, immunological or vascular injury of the joints. Of course, considering the highly heterogeneous nature of RA, it is probable that various insults can lead to joint inflammation (depending on the genetic background and lifestyle of the individual). ACPAs are unlikely to simply be immunological manifestations of joint inflammation, as they are RA-specific and are not detected in synovial tissue that is otherwise inflamed or infected, or in other degenerative diseases of the joints.

Despite all the evidence supporting an association between RA and periodontitis, the underlying molecular mechanisms have yet to be completely defined. To fully understand the clinical relationship and biochemical and immunological interplay between the two diseases, it is necessary to perform well-designed longitudinal, progressive multicentre clinical trials that enrol large numbers of very well-defined patients and controls. Importantly, such studies should adjust for known confounding factors such as drug use, differences in oral hygiene, socioeconomic status, age, sex and smoking habits. In addition, the immune response to *P. gingivalis* should be assessed by testing the levels of antibodies specific for major antigens expressed exclusively by this bacterium. Together with a standardized approach, this type of testing would avoid bias caused by antigen crossreactivity and antibody specificity.

Table 1 | **Summary of pivotal clinical, epidemiological and serological studies linking periodontitis and rheumatoid arthritis**

Study type	Study aim	Cohort	Study conclusions	Refs
Clinical	To examine the influence of periodontal conditions and microbiological status on rheumatological disease parameters in patients with rheumatoid arthritis (RA)	168 patients with RA and 168 healthy individuals matched for age, sex and smoking status	Although patients with RA had worse periodontal conditions than healthy individuals, the importance of periodontal pathogens in the periodontitis–RA interrelationship remains unclear	121
	To examine the effects of periodontal therapy on the clinical parameters of RA	60 patients with periodontitis and RA, 30 of whom received nonsurgical periodontal therapy	Routine periodontal therapy reduces the severity and symptoms of RA	122
	To evaluate the effects of routine periodontal therapy on the levels of tissue-type plasminogen activator and plasminogen activator inhibitor 2 in gingival crevicular fluid (GCF) of patients with periodontitis, with or without RA	15 patients with periodontitis and RA, 15 patients with periodontitis only and 15 matched healthy individuals	The plasminogen activating system is involved in periodontitis pathogenesis	123
	To determine whether serum immunity to *Porphyromonas gingivalis* peptidylarginine deiminase (PPAD) affects the clinical response to biologic DMARDs in patients with RA	60 patients with RA, who had retrospectively determined rheumatologic and periodontal conditions	Anti-PPAD antibody levels affect the clinical response to biologic DMARDs in patients with RA.	51
	To evaluate the periodontal condition of and level of *P. gingivalis* infection in individuals at risk of developing RA and in individuals with early RA	119 individuals at risk of developing RA, 48 patients with early RA and 167 age and sex matched healthy individuals	Infection with *P. gingivalis* and periodontal inflammation underpins the development of anti-citrullinated protein antibodies (ACPAs).	53
	Prospective follow-up study to investigate the link between RA and periodontitis in the context of the role of anti-rheumatic drugs in periodontal health	53 patients with early untreated RA, 28 patients with chronic RA who had previously not responded well to DMARDs and 43 age, sex and community matched healthy individuals	Periodontitis was more prevalent in patients with RA than in healthy individuals and RA treatment did not affect the severity of periodontitis	124
	To assess the effects of DMARDs and anti-TNF therapy on subgingival plaque microbiota and periodontal conditions in patients with RA	62 patients with RA being treated with anti-TNF and 115 patients being with RA treated with DMARDs	Different treatments for RA had variable effects on the clinical parameters of periodontitis and subgingival microbiota	125
	To examine the reciprocal relationship between the level of active matrix metalloproteinase 8 and periodontal pathogens in the GCF of patients with RA with varying periodontal conditions	103 patients with RA and periodontitis and 104 patients with RA and no periodontitis	RA seems to influence the host response in the periodontium of patients with periodontitis	126
	To evaluate the occurrence of citrullinated histone H3 in the inflamed periodontium and assess the level of anti-citrullinated histone antibodies in sera from patients with RA or periodontitis	113 patients with periodontitis, 84 patients with RA and 36 healthy individuals	Citrullinated histone H3 is present in the inflamed periodontium and could be a target for the development of autoantibodies	127
Epidemiological	To determine the association between periodontitis and *P. gingivalis* and the clinical and pathologic features of RA	287 patients with RA and 330 healthy individuals	Both *P. gingivalis* and periodontitis affected the autoimmune response in RA and there was an association between periodontitis and seropositive RA	45
	To verify if *P. gingivalis* mediates citrullination leading to the generation of RA-associated autoantibodies in genetically predisposed individuals	51 patients with generalized aggressive periodontitis, 50 patients with chronic periodontitis, 89 healthy individuals; all individuals were tissue-typed for the expression of RA-predisposing HLAs	• A relationship exists between periodontitis and RA • In patients with periodontitis, the presence of periodontal pathogens was not associated with anti-cyclic citrullinated protein antibodies or antibodies against citrullinated α-enolase	128
	A case–control examination of the prevalence of periodontitis in a well-characterized population-based Swedish cohort of patients with RA	2,740 patients with RA and 3,942 age, sex and residential area matched healthy individuals	Only smoking and ageing are risk factors for periodontitis, both in patients with RA and healthy individuals	46
	To analyze how the profile of antibodies produced against *P. gingivalis* lipopolysaccharide differs based on genetic factors, environmental factors and the severity of RA	694 patients with early RA, 61 patients with periodontitis, 54 patients with sicca symptoms and 79 healthy individuals	Tobacco smoking has such a strong effect on RA that the role of *P. gingivalis* in RA pathogenesis could only be seen in those who had never smoked	129

Table 1 (cont.) | **Summary of pivotal clinical, epidemiological and serological studies linking periodontitis and rheumatoid arthritis**

Study type	Study aim	Cohort	Study conclusions	Refs
Serological	To assess levels of matrix metalloproteinase 9 (MMP-9) as a potential biomarker for the association between RA and periodontitis	16 patients with active RA, 14 patients with periodontitis, 12 patients with RA and periodontitis and 21 healthy individuals	• The link between RA and periodontitis is associated with deregulation of the inflammatory reaction • MMP-9 is a potential tool for the diagnosis and management of patients with periodontitis and RA	130
	To evaluate the effects of routine periodontal therapy on GCF levels of matrix metalloproteinase 8, IL-6 and prostaglandin E2 in patients with RA and periodontitis	27 patients with gingivitis or periodontitis and RA, 26 patients with gingivitis or periodontitis alone and 13 matched healthy individuals	Routine periodontal therapy in patients with RA and periodontitis might provide beneficial effects on local inflammation	131
	To evaluate the antibody response to *P. gingivalis* virulence factor gingipain B in relation to ACPAs, smoking and *HLA-DRB1* shared epitope alleles, in patients with periodontitis or RA	65 patients with periodontitis and 59 non-periodontitis controls were selected separately from 1,974 patients with RA and 377 non-RA controls derived from the EIRA study	*P. gingivalis* is a sound candidate for initiating and/or driving autoimmunity and autoimmune disease in a subset of patients with RA	47
	To estimate the effect of periodontal therapy on serum levels of ACPA and PPAD in patients with RA	52 patients with RA (regardless of periodontitis status) and 26 healthy individuals	An association between anti-PPAD antibodies and ACPA responses suggests a role for PPAD in protein citrullination in patients with RA who have periodontitis	50
	To investigate whether the immune response to gingipain B and a citrullinated peptide derived from PPAD precedes RA symptoms	251 patients with RA and 198 matched healthy individuals	Increased anti-*P. gingivalis* antibody levels detectable years before the onset of symptoms of RA argues that *P. gingivalis* is involved in the development of RA	52
	To investigate associations between periodontitis and ACPA production in a healthy population	Nagahama study group, consisting of 9,554 healthy adults, including 6,206 non-smokers	Associations between periodontitis parameters and levels of ACPA in a cohort of healthy individuals support the essential contribution of periodontitis to ACPA production and the development of RA	54

Experimental evidence

Accumulating epidemiological and clinical data suggesting the involvement of periodontal pathogens, especially *P. gingivalis*, in the development of RA are directly supported by the results of animal studies (BOX 2). Despite differences in experimental design, the outcomes of such studies were consistent. The clinical symptoms of arthritis in mice and rats (the incidence and severity of swelling and erythema in the fore and hind paws) were exacerbated upon bacterial infection, accompanied by a massive influx of leukocytes, the accumulation of osteoclasts and cartilage and bone erosion[56–62]. The notable exception is *Prevotella intermedia*, which was tested in DBA1/J mice, in which no visible effect was noted[56]. In this model, subcutaneous chambers were inoculated with bacteria before the induction of collagen-induced arthritis (CIA). Similarly, there was no difference in the development or severity of pristane-induced arthritis between rats infected with periodontal pathogens and those not infected, even though pre-existing periodontitis was seemingly responsible for the production of antibodies against PPAD-derived citrullinated peptides in these animals[63].

The increased inflammatory reaction observed in infected mice was not limited to the joints and oral cavity; indeed, there was a systemic T_H17 cell response that might have affected the bones in the extremities[57]. Importantly, in another study, ACPAs were detected in animals exposed to wild-type *P. gingivalis*, but not in those infected with a PPAD-null strain[56]. Inoculating mice with CIA with red complex bacteria also facilitated dissemination of *P. gingivalis* to synovial joints[58]. The importance of IL-17 in linking experimental periodontitis and RA was confirmed in 2017 (REF. 64). Mice with antigen-induced arthritis (AIA) that had been orally infected with *P. gingivalis* had more severe joint damage ($P<0.05$) and higher frequencies of T_H17 cells ($P<0.05$) than non-infected mice with AIA. The aggravation of AIA induced by periodontitis was accompanied by increased levels of TNF and IL-17 and by increased infiltration of the joints by neutrophils, although no such observations were noted in mice deficient for IL-17 receptor A[64].

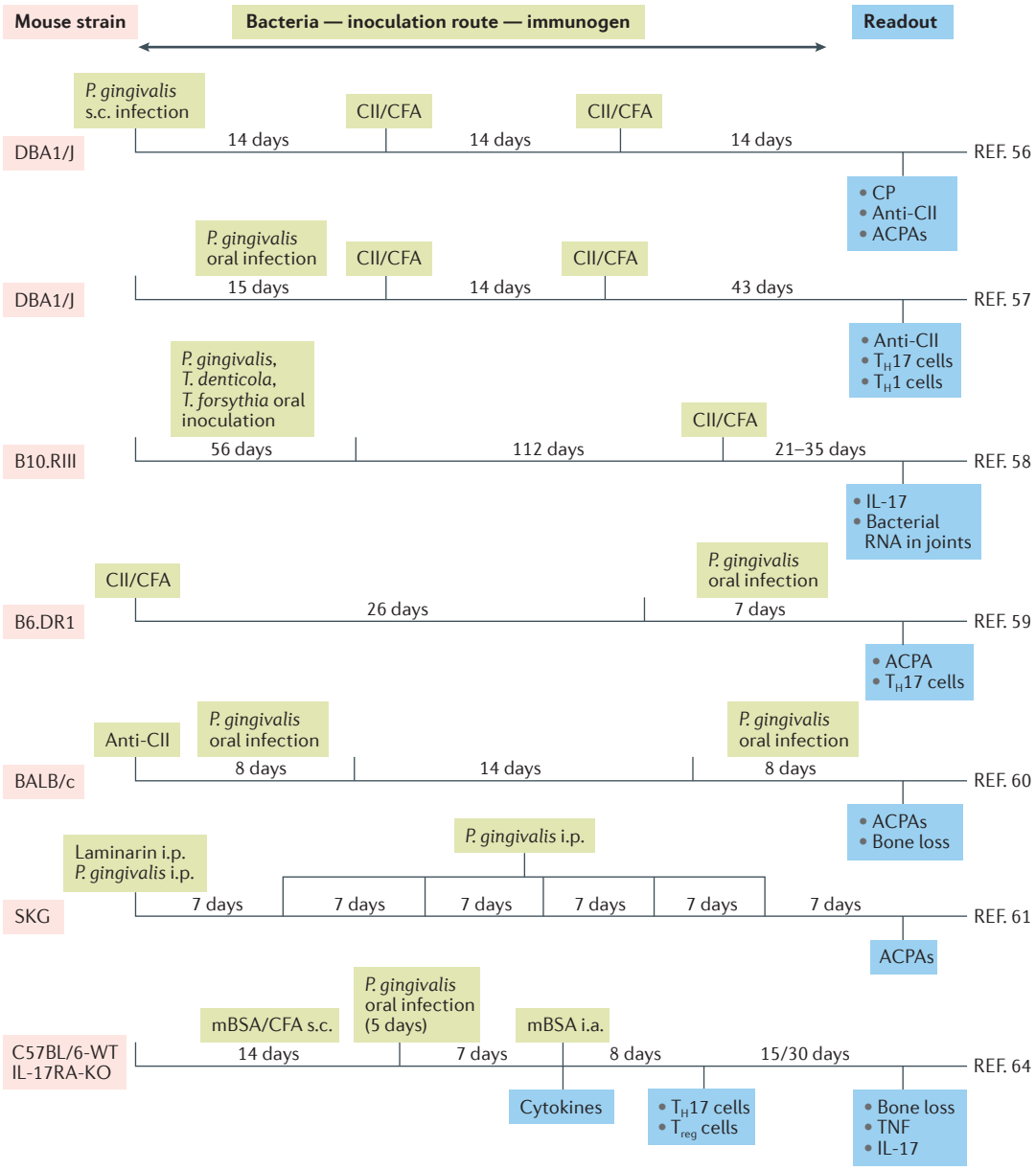
In 2017, the bidirectional interaction between periodontitis and RA seen in clinical and epidemiological studies (TABLE 1) was reproduced in the rat model of CIA[65]. In this study, periodontitis was induced by ligating the first molars on the right-hand side 3 weeks after the initial immunization of the rats with type II collagen and 1 week after a second local immunization with complete Freund's adjuvant in the paw[65]. In this experimental set-up, periodontitis increased the levels of rheumatoid factor in the serum and ACPAs in gingival tissue, as well as altering the cytokine balance in these animals. Interestingly, increased levels of IL-17 were found regardless of the presence of periodontitis, but only in those animals that developed RA. This type of experimental periodontitis significantly aggravated the

Box 2 | **Murine models used to investigate the role of periodontitis in rheumatoid arthritis pathogenesis**

Several different rodent models have been used to study the links between periodontitis and rheumatoid arthritis (RA), a selection of which are illustrated in the Figure. In the collagen-induced arthritis model, DBA1/J[56,57,62], B10.RIII (REF. 58) and HLA-DR1 humanized C57BL/6 mice (B6.DR1 mice)[59] were challenged with type II collagen (CII) to induce an immune response against the antigen. Alternatively, RA was induced in BALB/c mice by intravenous injection of monoclonal anti-CII antibodies[60], local injection of methylated bovine serum albumin into the knee joint of previously immunized animals[64] and in rats by intradermal injection of pristane[63]. SKG mice are characterized by a single point mutation in the *Zap70* gene[61]. The mutation in *Zap70* attenuates T cell receptor signalling and causes abnormal selection of T cells. In this model, autoimmune RA is induced by activated autoreactive $CD4^+$ T cells that have escaped negative selection.

In these models of RA, the animals were inoculated orally, subcutaneously (s.c.) or intraperitoneally (i.p.) with periodontal pathogens, including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *P. gingivalis* with *Fusobacterium nucleatum*, or with a polybacterial mixture of red complex bacteria (*P. gingivalis*, *Tannerella forsythia* and *Treponema denticola*), either before, at the same time as or after induction of arthritis. In the case of *P. gingivalis*, the wild-type parental strain or the isogenic peptidylarginine deiminase-null mutant were used.

ACPAs, anti-citrullinated protein antibodies; Anti-CEP1, antibodies against peptide 1 of citrullinated α-enolase; Anti-CII, antibodies against type II collagen; CFA, complete Freund's adjuvant; CP, citrullinated proteins; i.a., intra-articular; IL-17RA-KO, interleukin-17 receptor A knock out; mBSA, methylated bovine serum albumin; T_H1 cells, T helper 1 cells; T_H17 cells, T helper 17 cells; T_{reg} cells, regulatoryT cells; WT, wild type.



pathological read-out of RA (P <0.5) and, conversely, the presence of RA increased periodontal destruction[65]. Notably, ligature-induced periodontitis in rats depends on the development of a dysbiotic bacterial biofilm from natural commensal microbiota, which become enriched with *P. gingivalis*-like species[66], thereby more closely mimicking human periodontal infection than other murine models of periodontitis.

Taken together, these data suggest that infection by *P. gingivalis* promotes the development of and affects the severity of arthritis, at least in rodent models of RA. These results highlight mechanistic aspects of the intimate relationship between RA and periodontitis. The PPAD-dependent breakdown of immune tolerance caused by *P. gingivalis* can be considered the causative link between periodontal infection and RA; however, endogenous PADs are also important since they are highly active in chronic infections such as periodontitis. Moreover, in a study using SKG mice, which spontaneously develop chronic autoimmune arthritis[67], expression of modified proteins was increased in the tissues of the ankle joint, but not in the serum, following systemic injection of *P. gingivalis*[61]. The accumulation of high levels of citrullinated proteins, including peptides bearing C-terminal citrulline residues, in inflamed tissues, increases the possibility of such proteins being recognized as foreign antigens by the immune system. In this manner, systemic infection with *P. gingivalis* can affect the local environment within the joint.

A study from 2016 in humans partially confirms the conclusions drawn from these studies in experimental models. A single-cell antibody cloning approach revealed that plasmablast-derived antibodies from ACPA-positive patients with RA, but not from healthy individuals or patients with ACPA-negative RA, react specifically with *P. gingivalis*-derived citrullinated antigens, including citrullinated α-enolase[68]. These fascinating results suggest that some ACPAs can be generated by the immune response to *P. gingivalis*. Overall, although data from experimental models confirm the relevance of periodontal pathogens in RA pathogenesis, we are still far from understanding the role of individual periodontal pathogens in the breakdown of immune tolerance that underlies the generation of ACPAs that precipitate and propagate RA.

Mechanisms linking periodontitis to RA

Microbial involvement in the aetiology of RA was first suggested in the nineteenth century; however, it is only in the past few years that ground-breaking discoveries have shed light on how bacterial dysbiosis at mucosal surfaces can trigger a chain of events that lead to full-blown RA[16]. A growing body of evidence indicates that dysbiosis in the microflora of the gut and gums could be major factors (in addition to cigarette smoke) contributing to the initiation and progression of RA. *P. gingivalis*, the major pathogen involved in chronic periodontitis, and *A. actinomycetemcomitans* seem to have key mechanistic roles. *P. gingivalis* can effectively deregulate local immune responses in the periodontium and both directly and indirectly affect the inflammatory status of the joints, whereas, in the case of *A. actinomycetemcomitans*, bacterially secreted leukotoxin is directly responsible for releasing the hypercitrullinated cargo from neutrophils at sites of periodontitis, and the titre of antibodies specific for the bacterium (and the toxin itself) is strongly associated with the presence of ACPA and rheumatoid factor in patients with RA[34]. In the following section, we describe the main molecular pathways involved in these processes (FIG. 3).

Chronic inflammation

The most popular theory linking periodontitis to RA is based on the shared pathophysiology between the two diseases. Bacterial biofilms in the form of dental plaques are responsible for maintaining chronic inflammation in the periodontium[11,12]. This accumulation of bacteria causes the activation of Toll-like receptors (TLRs) on immune cells and the recognition of pathogen-associated molecular patterns (PAMPs) by gingival epithelial cells and resident phagocytes, inducing the localized secretion of pro-inflammatory cytokines such as IL-1β, TNF, IL-6 and IL-8 (REF. 69) (FIG. 3). This chronic immune response has systemic consequences, which manifest as an increased production of CRP and high levels of pro-inflammatory cytokines in the serum of patients with periodontitis[13]. Among periodontal pathogens, *P. gingivalis* possesses a diverse range of PAMPs, including lipopolysaccharide (LPS), fimbriae and gingipains (proteolytic enzymes that cleave at arginine residues), which ensure the activation of a broad range of immune receptors such as TLR2, TLR4, nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and proteinase-activated receptor 2 (PAR2), and explain the dominant role of *P. gingivalis* in the development of inflammation[70]. Dysregulation of the cytokine network and aberrant activation of leukocytes participating in the innate immune response to periodontal pathogens could therefore be another explanation for the clinical and epidemiological link between periodontitis and RA (FIG. 3).

The role of T cell differentiation as the molecular link between RA and periodontitis has been well documented. T_H17 cell-related cytokines are strong inducers of arthritis, and IL-17 is important for osteoclast differentiation and the development of bone erosions. The number of T_H17 cells was known to increase in the early and active stages of RA, but the underlying mechanism between this increase and periodontitis was unclear. A 2016 study utilizing the murine CIA model revealed that oral infection with *P. gingivalis* preceding the induction of arthritis favoured a T_H17 cell-driven response in the serum via IL-17 and IFNγ, which ultimately affected disease development and progression[58]. Periodontal pathogens such as *P. gingivalis* and *Prevotella nigrescens*[62] induced the differentiation of T_H17 cells in an IL-1-dependent manner by activating antigen-presenting cells in the murine CIA model; these pathogens also induced T helper 1 (T_H1) cell responses by directly activating TLRs on T cells[62]. IL-1β is crucial for T cell differentiation, as confirmed in mice

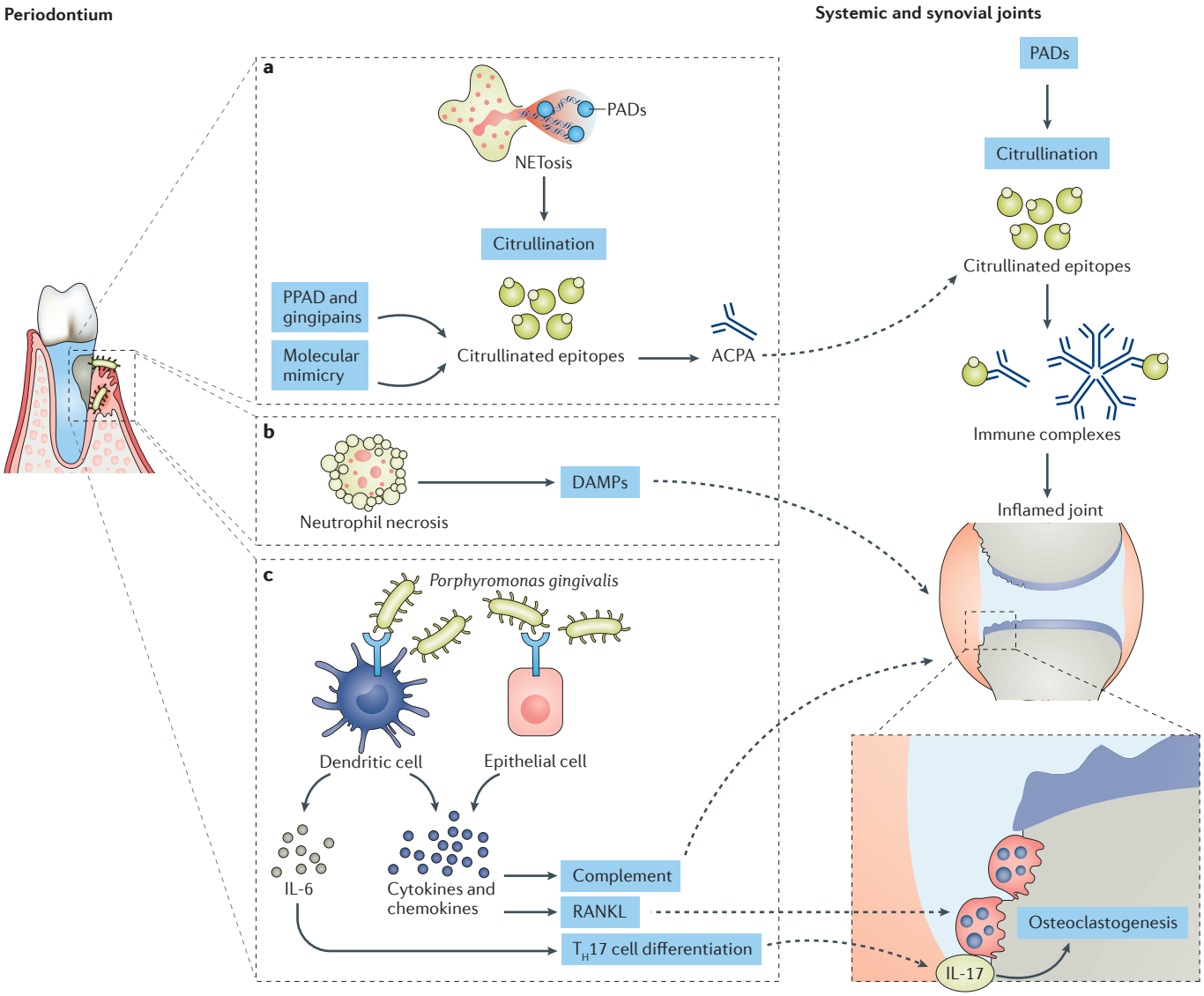


Figure 3 | **Proposed mechanisms underlying the links between periodontal disease and the pathogenesis of rheumatoid arthritis.** **a**| In response to *Porphyromonas gingivalis* infection, neutrophils can release neutrophil extracellular traps (NETs), structures characterized by active proteases and peptidylarginine deiminases (PADs). The concomitant action of these enzymes generates citrullinated epitopes and triggers the synthesis of anti-citrullinated protein antibodies (ACPAs). The production of citrullinated epitopes is accelerated by the synergistic action of gingipains and *P. gingivalis* peptidylarginine deiminase (PPAD), both of which are unique to *P. gingivalis*. Molecular mimicry by some bacterial proteins (such as bacterial enolase with human α-enolase) is also involved in the breakdown of immune tolerance to host molecules. A secondary signal directed against citrullinated epitopes in the joints leads to increased production of rheumatoid factor and ACPAs, leading to the accumulation of immune complexes. **b**| Neutrophils attracted to the gingival crevice undergo necrosis, thereby releasing damage-associated molecular patterns (DAMPs), which accelerate local and systemic inflammation. **c**| In the infected periodontium, virulence factors expressed by *P. gingivalis*, such as lipopolysaccharide, fimbriae, gingipains and lipoproteins, are recognized by Toll-like receptors, protease activated receptors and/or nucleotide-binding oligomerization domain-containing 2 (NOD2) receptors on gingival epithelial cells and phagocytes, such as dendritic cells. In response to pathogens, the host cells release cytokines (such as IL-6) and chemokines that activate the complement system, receptor activator for nuclear factor-κB ligand (RANKL) signalling pathways and the differentiation of T helper cells, which contribute towards osteoclastogenesis.

devoid of IL-1 receptor antagonist, which are prone to autoimmune diseases[71]. Moreover, IL-1 has a pivotal role in bone erosion, activating the nuclear factor-κB (NF-κB)–receptor activator of NF-κB ligand (RANKL; also known as TNFSF11) pathway, which leads to the activation of osteoclasts[72]. The balance between T_H1 cells, T_H2 cells and T_H17 cells is also extremely important in controlling the immune events that lead to bone destruction[73]. Interestingly, pro-inflammatory cytokines such as IL-1 and TNF amplify the effects of IL-17 (REF. 73).

ACPAs

Chronic inflammation impairs the immune system, thereby creating an environment ideally suited to the breakdown of immune tolerance. Increased amounts of

damage to cells in inflamed tissue, accompanied by high concentrations of calcium ions, promotes the activity of PADs. This enzyme activity, combined with the release of intracellular proteins, results in the rapid and uncontrolled generation of citrullinated epitopes, which in turn trigger autoimmune responses via the binding of ACPAs (FIG. 3). This mechanism provides the molecular basis for linking periodontitis to RA. ACPAs generated in the gingiva of patients with periodontitis are thought to react with citrullinated peptides in the synovia, which might be formed after a traumatic event in the joint[74]. In this scenario, the citrullination of autoantigens in synovial tissue and at other mucosal sites, such as the inflamed gingiva, is a prerequisite for the initiation and maintenance of autoimmune reactions in patients with RA. Since the generation of citrullinated epitopes depends upon PADs, their importance in the generation of the autoantigens that drive autoimmunity in RA is unquestionable. In this context, it is important to remember that PAD2 and PAD4 (and their products) are detected together with ACPAs in the inflamed gingiva and in the gingival crevicular fluid (GCF) of patients with periodontitis[75].

Remnant epitopes

A role for antigen modification is suggested by the remnant epitope generates autoimmunity (REGA) model[76]. This model is based on the orchestrated action of cytokines and proteases secreted by phagocytes, which result in the extracellular proteolytic degradation of intact proteins into remnant fragments that often contain immunodominant epitopes, known as remnant epitopes. In the case of RA, intra-articular neutrophils attracted by IL-8 secrete neutrophil collagenase (also known as MMP-8) and matrix metalloproteinase 9 (MMP-9), which then catalyse the degradation of collagen[77]. GCF from patients with periodontitis exerts strong proteolytic activity[78], making the generation of remnant epitopes, which might induce an autoimmune reaction, a distinct possibility[79]. To this end, it is interesting to note that lysine-specific gingipain cleaves human IgG *in vivo* to release Fab fragments[80], which are recognized as neo-epitopes by anti-hinge autoantibodies[23].

Molecular mimicry

The link between periodontitis and RA can also be explained by molecular mimicry (FIG. 3). This idea is based on the observation that some antigens expressed by *P. gingivalis* are structurally similar to self-antigens, and can therefore crossreact with ACPAs. The strongest candidate for such mimicry is bacterial enolase, which shares 51.4% amino acid identity with its human orthologue, α-enolase[81]. Citrullinated bacterial enolase in inflamed periodontal tissue could trigger an immune response, leading to the generation of antibodies that recognize peptide 1 of citrullinated human α-enolase (anti-CEP1 antibodies), which shares a homology of 82%[81]. Citrullinated *P. gingivalis* enolase crossreacts with ACPAs; the potential pathogenic role for this protein was verified in a DR4-IE transgenic mouse model of arthritis[82]. Immunizing mice with bacterial enolase also induces synovial hyperplasia and erosions, with the appearance of anti-CEP1 antibodies[82].

Another candidate for molecular mimicry is heat shock protein 60 (Hsp60) from *P. gingivalis*. A comparison of Hsp60 from *P. gingivalis*, *Mycobacterium tuberculosis* and *Chlamydia pneumoniae* revealed that only Hsp60 derived from the periodontal pathogen contained a peptide epitope that is predominantly and consistently recognized by antibodies from the serum of patients with RA[83]. The above bacterial molecule is highly crossreactive with human antibodies, suggesting that similarities between antigens expressed by oral bacteria and humans could be important in the pathogenesis of RA[83]. Notably, many other types of bacteria found in dental plaques also express enolase and Hsp60, which are highly conserved, so could contribute to molecular mimicry and the breakdown of immune tolerance in inflamed gingival mucosae.

PPAD

The strongest evidence linking periodontitis with RA came from the discovery of PPAD, which is uniquely expressed by *P. gingivalis*. PPAD strongly influences the immune system and can induce the production of autoantigens that drive autoimmunity in RA[84]. PPAD activity, in conjunction with that of gingipains generates peptides and protein fragments that are citrullinated at their C-terminus, which might represent epitopes that are new to the immune system (FIG. 4). Interestingly, human PADs cannot deiminate C-terminal arginine residues[85]. Among the host proteins involved in the development of RA, fibrinogen, α-enolase and vimentin are all sources of C-terminally citrullinated peptides when exposed to gingipains or PPAD[9]. Notably, both PPAD[86] and gingipains[87] are detectable in GCF, indicating that these enzymes have access to substrates such as fibrinogen and α-enolase that are relevant for the development of autoantibodies. PPAD activity in the periodontium is also increased in both patients with RA and individuals who do not have RA, but do have periodontitis[86]. The synchronized action of PPAD and gingipains fits well into the REGA model, supporting a role for periodontitis during the development of autoimmune diseases. Moreover, citrullinated PPAD might directly contribute to the breakdown of immune tolerance[84]. However, this pathway does not seem to be important since PPAD produced by *P. gingivalis* does not undergo autocitrullination (in contrast to the recombinant full-length enzyme expressed in *Escherichia coli*), and anti-PPAD antibodies from patients with RA are exclusively directed against the unmodified enzyme[88].

The role of PPAD as a virulence factor of *P. gingivalis* is not limited to neoepitope generation; the activity of this enzyme can modulate inflammation and homeostasis in the infected periodontium by citrullinating the C-terminal arginine residues of bradykinin[89], anaphylatoxin C5a[90] and epidermal growth factor[91], as well as contributing to prostaglandin secretion in infected fibroblasts[92]. In this way, PPAD might directly enhance the severity of periodontitis in humans, as observed in the rodent model of the disease[60]. Taken together, these data suggest that PPAD is a potent and versatile virulence factor expressed by *P. gingivalis*, the activity of

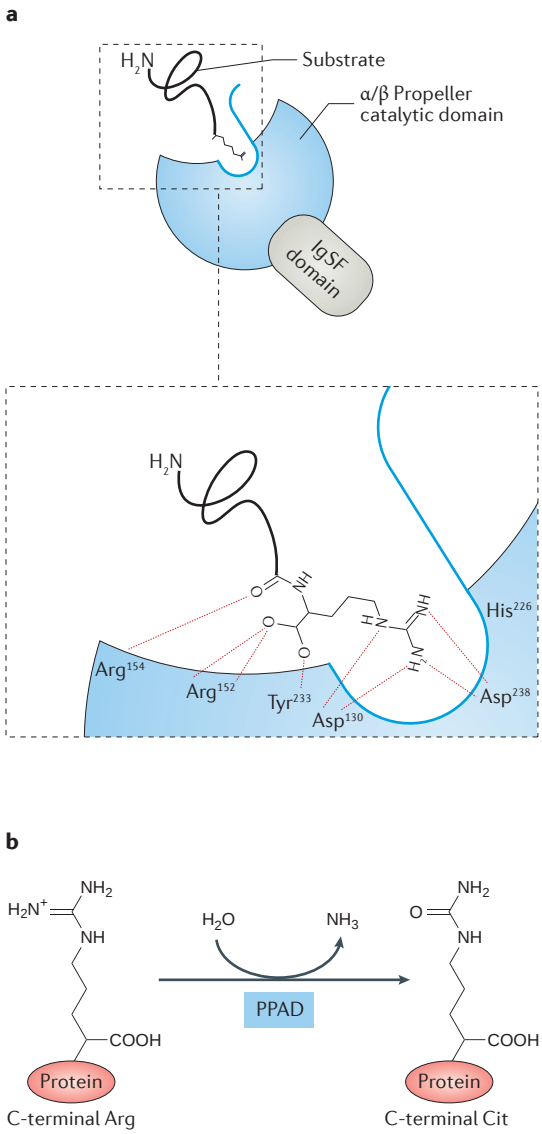


Figure 4 | **C-Terminal protein citrullination catalysed by *Porphyromonas gingivalis* peptidylarginine deiminase (PPAD). a|** The molecular architecture of PPAD generally resembles that of human peptidylarginine deiminases (PADs) 2 and 4, with the molecule consisting of an α/β propeller catalytic domain followed by an Ig-like domain (IgSF). However, in PPAD, a steric hindrance of protruding loops surrounding the active site cleft (shown as a thick blue line) limit interactions between the substrate and the main chain of the reactive site cleft to only one side of a deiminated arginine side chain, while the arginine guanidine group interacts with the catalytic residues of PPAD (Asp_{130}, Asp_{238} and His_{226})[120]. In addition, the carboxylate of the C-terminal arginine and the carbonyl of the preceding peptide bond are firmly anchored by salt bridges and hydrogen bonds to residues (Arg_{152}, Arg_{154} and Tyr_{233}) lining the substrate-binding cleft of PPAD[120]. **b|** These differences in substrate binding between PPAD and human PADs mechanistically explain why PPAD has a strong preference for C-terminal arginine residues. PPAD catalyses a reaction in which a guanidine group is hydrolysed, releasing ammonia and forming a C-terminal citrulline residue.

which in inflamed epithelium might provide direct (via the generation of citrullinated epitopes) or indirect (via enhanced inflammation of the periodontium) mechanistic links between periodontitis and RA.

Other potential mechanisms

The aetiology of RA is complex and the list of molecular pathways linking periodontitis to RA outstrips our current knowledge. In this section, we describe some mechanisms that have been put forward in the past few years, but have not yet received strong experimental support.

NET generation by neutrophils. One potential mechanism involves neutrophils; specifically, the excessive generation of NETs, structures made from DNA, histones, the contents of intracellular granules and antimicrobial peptides that form an extracellular matrix-like structure. Although the antimicrobial function of NETs is beneficial, uncontrolled NET formation or delayed clearance of NETs is associated with several autoimmune diseases[93,94], including RA. Neutrophils from patients with RA have a greater propensity to produce NETs than those from healthy individuals; increased NET production is also associated with more severe disease activity in patients with RA[95]. Accumulated NETs might contribute to tissue damage and provide a source of autoantigens; indeed, ACPAs from the serum of patients with RA react with histones found in NETs[95] (FIG. 3).

The primary immunogenic role of NETs is associated with the activity of PAD4, which catalyses the citrullination of histones and other molecules such as α-enolase, potentially leading to a breakdown of immune tolerance. NETs might also amplify the autoimmune reaction in joints, since the binding of NETs to ACPAs creates immune complexes that subsequently induce further NET release by binding to Fcγ receptor[95,96]. Large numbers of neutrophils are found within the inflamed gingiva; these cells are hyper-reactive in terms of ROS release and are prone to release NETs in response to periodontal pathogens[97]. Differences in NET generation between individual patients with periodontitis are relatively large; nevertheless, NETs are present in dental plaque samples, including GCF and saliva[98]. Increased NET production and/or impairment of NET clearance in patients with periodontitis suggest that NETs are generated during chronic gingival infection and might provide a source of autoantigens, which could lead to RA.

Damage-associated molecular patterns. Another possible mechanism is the inhibition of the phagocytosis of apoptotic cells. Engulfment of apoptotic cells by phagocytes activates immunosuppressive pathways and triggers the production of anti-inflammatory cytokines that prevent immune responses against self-antigens[99]. Conversely, failure to clear apoptotic cells leads to secondary necrosis and the release of cellular contents, including noxious intracellular molecules, which subsequently engage receptors for damage-associated molecular patterns (DAMPs)[100]. Activation of DAMP

signalling pathways might break tolerance to self-antigens, thereby contributing to autoimmune diseases such as RA (FIG. 3).

One group of DAMPs, the alarmins, are endogenous molecules that are rapidly released into the extracellular milieu, either passively by necrotic cells or actively by immune cells[101]. Well-known alarmins thought to be important in the development of RA include heat shock proteins, hyaluronan, uric acid, high mobility group box protein 1 (HMGB1), S100 proteins, IL-1α and IL-33, some of which have been identified as factors that promote the development of periodontitis[102]. In particular, HMGB1 is released into the extracellular space by macrophages and necrotic gingival epithelial cells, whereupon it increases the expression of adhesion molecules by dendritic cells, T cells and endothelial cells[103]. In the presence of RANKL, HMGB1 can also influence the differentiation of osteoclast precursors into osteoclasts[103]. These data suggest the involvement of HMGB1 in periodontitis, as LPS can stimulate the release of HMGB1 from macrophages. Indeed, the concentration of HMGB1 in GCF and the number of HMGB1-positive cells present in the inflamed gingival epithelium of patients with periodontitis is higher than that of healthy individuals[104].

IL-33 is another potential link between periodontitis and RA[101]. IL-33 accumulates intracellularly, but is released into the extracellular milieu upon cell damage. Binding of IL-33 to IL-1 receptor-like 1 activates NF-κB and mitogen-activated protein kinase pathways, causing the induction of pro-inflammatory immune responses and cytokine production[105], and IL-33-mediated signalling leads to bone erosion in experimental models of arthritis[106]. IL-33 has also been detected in GCF from patients with chronic periodontitis and *P. gingivalis* has been identified as a pathogen that can increase expression of this molecule in periodontal tissue[102,107]. The role of IL-33 in periodontitis was finally confirmed by the observation of RANKL-dependent *P. gingivalis*-exacerbated alveolar bone loss after IL-33 administration in the murine model of periodontitis[102].

Taken together, an increasing body of evidence suggests that alarmins are involved in the pathogenesis of both RA and periodontitis. Indeed, one might suggest that systemic release of DAMPs should be considered as yet another possible molecular mechanism linking both diseases.

Conclusions

The body of evidence built up over the past decade leaves little doubt that periodontitis and RA are intimately linked (FIG. 3), and that these links are not just due to similarities in pathogenic mechanisms and shared environmental and genetic risk factors. Furthermore, data from large cohort studies showing that periodontitis precedes the development of RA, and that periodontitis in individuals who later go on to develop RA positively correlates with ACPA levels, strongly argue for a causative relationship. In this model, the chronically inflamed periodontium is the site at which immune tolerance to citrullinated epitopes is broken and the production of ACPAs begins. This theory has been verified in animal models of periodontitis and RA, and is in line with the paradigm that ACPAs are generated at the mucosal surfaces and that the generation of ACPAs precedes the clinical symptoms of RA by many years.

Many of the mechanisms underlying hypercitrullination under inflammatory conditions (a prerequisite for tolerance breakdown) operate at mucosal surfaces. In the case of chronic inflammation of the periodontium, nearly all the mechanisms that contribute to the formation of citrullinated epitopes are driven by periodontal pathogens. In this situation, periodontal pathogens can be considered to be a direct trigger for subsequent autoimmune reactions that underpin diseases such as RA. The mechanistic effect of periodontitis and periodontal pathogens in the development of RA is exemplified in case–control studies showing that ACPAs are generated at other mucosal surfaces such as the lungs and gut, providing a tightly interwoven network of interactions that can trigger the initiation of RA.

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Differential antibody glycosylation in autoimmunity: sweet biomarker or modulator of disease activity?

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Abstract | A loss of humoral tolerance is a hallmark of many autoimmune diseases and the detection of self-reactive antibodies (autoantibodies) of the immunoglobulin G (IgG) isotype is widely used as a biomarker and diagnostic tool. However, autoantibodies might also be present in individuals without autoimmune disease, thus limiting their usefulness as a sole indicator of disease development. Moreover, while clear evidence exists of the pathogenic effects of autoantibodies in mouse model systems, the contribution of autoantibodies to the pathology of many autoimmune diseases has yet to be established. In this Review, the authors discuss the changes in total serum IgG and autoantibody glycosylation that occur during autoimmune disease and how these changes might help to predict disease development in the future. Furthermore, current knowledge of the signals regulating antibody glycosylation and how individual antibody glycoforms could be used to optimize current treatment approaches will be discussed.

Chronic inflammatory and autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and inflammatory bowel disease affect millions of people worldwide. Several novel innovative treatment options have become available, such as anti-CD20 antibodies or the neutralization of pro-inflammatory cytokines, although many patients do not respond to, or become resistant to such therapies[1,2]. Therapeutic interventions early in the course of disease can prolong the duration of treatment intervals and prevent, or delay the occurrence of severe tissue damage[3]. To enable such preventive approaches or early intervention, advanced diagnostic tools, such as biomarkers that enable disease development to be predicted, are needed. In many autoimmune diseases the loss of humoral tolerance and the appearance of autoantibodies specific for a variety of self-antigens is an established hallmark of disease. Research involving a variety of animal models has provided clear evidence that such autoantibodies can indeed induce autoimmune pathology through activation of cellular immunoglobulin-γ Fc receptors (FcγRs) expressed on a wide variety of innate and adaptive immune cells, or via the production of the activated complement components C3a or C5a (REFS 4,5). In the majority of human chronic inflammatory and autoimmune diseases, however, a direct contribution of specific autoantibodies to tissue damage has yet to be established. Autoantibodies can also be present in individuals without a history of chronic inflammatory or autoimmune disease and do not always correlate with disease activity, therefore, they are rarely used as standalone diagnostic tools[6,7]. Notable exceptions to this general rule include immune thrombocytopenia (ITP) and autoimmune haemolytic anaemia (AIHA), in which anti-platelet and anti-red blood cell antibodies, respectively, directly cause the autoimmune phenotype. Nonetheless, evidence exists in certain other diseases, such as RA and granulomatosis with polyangiitis (GPA), that autoantibodies specific for citrullinated proteins (ACPAs) or leukocyte proteinase 3 (PR3, also known as myeloblastin) might have a direct role in disease pathology[8–10]. However, further research is necessary to understand the underlying mechanisms in greater detail.

The appearance of autoantibodies can predate the development of disease symptoms by many years, although the mere presence of autoantibodies does not correlate with autoimmunity, suggesting that, in addition to the specificity of the autoantibody, other factors must determine the activity[11]. The isotype of the autoantibody might be one such factor, with the presence of IgG autoantibodies being associated with the development of autoimmune disease, whereas the presence of naturally occurring IgM autoantibodies might provide some protection from the development of disease through clearance of apoptotic and/or dying cells[12]. Apart from antibody isotype and subclass, the sugar moiety

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Key points

- Serum and/or autoantibody glycosylation is altered in many autoantibody-dependent and autoantibody-independent autoimmune diseases
- IgG glycosylation is a key regulator of (auto)antibody activity
- IgG glycovariants lacking galactose and sialic acid residues can appear before the onset of disease symptoms
- IgG sialylation impairs pro-inflammatory antibody activity and might also explain why autoantibodies can be present in patients with inactive, or no disease
- A high level of IgG galactosylation and sialylation can confer active anti-inflammatory activity
- Distinct sets of Fc receptors are involved in pro-inflammatory and anti-inflammatory antibody activity

attached to the IgG constant heavy 2 (C_H2) domain is critical for maintaining both the pro-inflammatory and anti-inflammatory effector functions of the IgG[13,14]. This 'Achilles heel' of IgG has been exploited therapeutically by administration of enzymes that deglycosylate the IgG Fc domain. Such treatment approaches have been shown to ameliorate the pathological signs of autoimmune diseases in a variety of preclinical model systems, including models of skin-blistering diseases, SLE and RA[15–17]. Furthermore, specific sugar residues, such as fucose, galactose and sialic acid have been shown to modulate the pro-inflammatory and anti-inflammatory effects of IgGs, which has led to a wealth of studies attempting to investigate how the glycosylation of total serum and autoreactive IgGs changes during the different phases of various autoimmune diseases. The aim of this Review is to discuss how certain sugar moieties influence the activity of IgGs, if, and how such moieties might be associated with different types of autoimmune diseases and whether these sugars could be used as biomarkers to predict the future development of autoimmune diseases.

IgG glycosylation as a biomarker

The sugar moiety attached to each of the asparagine 297 residues of the two IgG C_H2 domains consists of a heptameric biantennary structure, and is a critical determinant of the pro-inflammatory or anti-inflammatory effects of IgG[4,14] (FIG. 1). The constant core of this sugar domain consists of *N*-acetylglucosamine (GlcNac) and mannose residues, which might contain an additional bisecting GlcNac residue linked to either the first mannose, terminal galactose or sialic acid residues[13]. Furthermore, a branching fucose residue is attached to the first GlcNac residue in the majority of IgG-associated sugar moieties (FIG. 1a). The variable presence of terminal and/or branching sugar residues, asymmetrical glycosylation (the composition of the two sugar moieties attached to each Fc domain can vary), the presence of additional glycosylation sites introduced in the antibody variable region during somatic hypermutation, and a differential level of glycosylation among the four IgG subclasses creates a daunting level of complexity, which results in several hundred differentially glycosylated IgG variants being present in any individual person at any given time[13,18–20]. Despite the existence of age-associated changes in IgG glycosylation, this abundance of different glycoforms remains conserved in individuals without autoimmune diseases, enabling the identification of deviations from this stable glycosylation state to be identified in patients with autoimmune disease[13,21–23]. The original observations that serum IgG glycosylation is skewed towards moieties of the G0F glycoform, which lack terminal sialic acid and galactose residues but contain a branching fucose residue, in patients with RA or Crohn's disease, or in those with SLE and Sjögren syndrome date back to the 1970–1980s[24–26] (BOX 1). The findings of these early studies also showed that the relative abundance of G0F glycoforms increases during pregnancy or chronic infection, establishing that the IgG glycome is not static but can change under certain physiological conditions[13]. Over the past 20 years, many groups have confirmed these findings, suggesting that changes in IgG glycosylation might, in future, be a useful biomarker of chronic inflammatory and/or autoimmune diseases[27–34]. However, a major unanswered question remains as to whether these changes in IgG glycosylation are merely an effect of the inflammatory milieu, without any functional consequences for autoantibody activity, or whether they could provide a better understanding of the disease process and possibly even be responsible for the initiation and resolution of inflammation. In this Review we focus on the role of fucose, galactose and sialic acid residues, for which the most convincing level of evidence exists indicating an ability to modulate IgG activity, with alterations in the regulation of these residues during autoimmune responses.

Fucosylation and autoantibody activity

The majority (>90%) of IgG glycoforms in serum samples from mice or humans contain branching fucose residues[13,35]. Research from several groups investigating the activity of cytotoxic antibodies has demonstrated that IgG glycovariants lacking branching fucose residues have greatly increased levels of affinity for specific FcγRs, both in mice (FcγRIV) and in humans (FcγRIIIa/IIIb)[36–38] (FIG. 2). This finding was the first demonstration that the presence, or absence of individual sugar moieties can modulate the interactions of IgG with specific FcγRs, suggesting that this effect might also apply to other IgG glycovariants. This observation offered a potential explanation for the conundrum regarding the presence of autoantibodies without any evidence of pathological effects. However, in contrast to the strongly reduced levels of galactosylated and sialylated IgG glycoforms during active autoimmune disease, the abundance of fucosylated IgG glycosylation variants seems to remain stable during inflammation and vaccination in mice and humans[39–44]. In fact, the identity of the extrinsic factors that determine whether or not a B cell or plasma cell produces fucosylated or nonfucosylated IgG antibodies remains unclear. One group of researchers even reported an increase in the abundance of serum IgG fucosylation in patients with SLE over time[44]. Two alloantibody-mediated diseases of the newborn, the so-called fetal and neonatal alloimmune thrombocytopenia (FNAIT)[45–47] and haemolytic disease of the fetus and newborn (HDFN) provide notable exceptions, in which dramatic changes in IgG

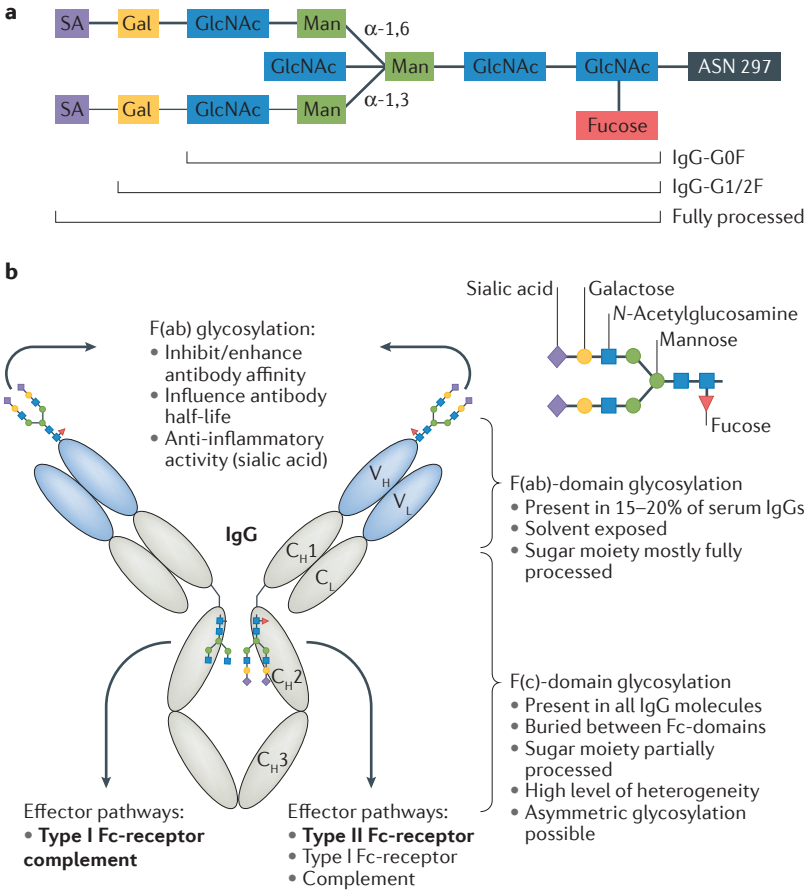

Figure 1 | **Effects of IgG glycosylation on antigen recognition and effector functions. a** | Schematic structures and individual components of the sugar moiety that can be attached to the IgG variable, or the Fc domain. Sugar residues are colour coded according to guidelines provided by the consortium for functional glycomics[127]. The sugar moiety is referred to either as IgG–G1F or IgG–G2F, depending on the presence of one, or two galactose residues. Sugar moieties without any terminal sialic acid (SA) and galactose (Gal) residues are referred to as IgG–G0F glycoforms. The fully processed sugar moiety contains terminal galactose and sialic acid residues on both arms. **b** | Sugar moieties attached to the IgG–Fc domain are rarely fully processed and can differ in composition within different constant heavy 2 (C_H2) domains of the same IgG molecule. Certain sugar moieties, such as fucose, galactose and sialic acid are able to influence IgG effector functions. Certain IgG molecules are glycosylated in the antibody variable (Fab) region, owing to the generation of glycosylation sites during somatic hypermutation. Fab glycosylation can have positive or negative implications for antigen binding and antibody half-life, and might also have immunomodulatory effects. C_H, constant heavy; C_L, constant light; GlcNAc, *N*-acetylglucosamine; Man, mannose; V_H, Variable heavy; V_L, Variable light.

fucosylation levels have been described[48]. In these two diseases, the mother develops antibodies against platelet or red blood cell associated antigens of the fetus, which can trigger severe thrombocytopenia or haemolytic anaemia in the fetus and/or newborn. Consistent with other autoimmune diseases, the mere presence of an autoantibody does not predict disease severity, which limits the value of these alloantibodies as biomarkers. In 2014, an analysis of IgG glycosylation patterns[48] revealed that many platelet-specific and rhesus factor D (RhD)-specific alloantibodies were afucosylated in pregnant women with HDFN or FNAIT, resulting in greater affinity for FcγRIIIa and FcγRIIIb. More importantly, the presence of afucosylated alloantibodies predicted disease severity in both diseases[46,48,49]. Investigations of alloresponses in patients with HDFN have also demonstrated that the low level of fucosylation is specific to responses to RhD, and that alloantibodies directed against other red blood cell antigens did not have this low-fucose glycosylation pattern, suggesting that the antigen itself might influence the overall effect of antibody glycosylation[49]. Taken together, IgG fucosylation is generally stable in a variety of autoimmune diseases, whereas a robust association exists between disease severity and the extent of antibody fucosylation during pregnancy. Thus, FNAIT and HDFN provide prime examples of differentially glycosylated autoantibodies as both biomarkers and active components of disease pathology. Why certain alloantigens induce antibody responses that generate afucosylated IgG glycoforms is unclear, but such knowledge might enable the identification of factors determining the extent of antibody fucosylation in B cells.

Changes in antibody galactosylation

A decrease in the level of IgG galactosylation (and hence an increase in the abundance of the G0F glycoform) is one of the most prominent and established changes in IgG glycosylation, appearing at the level of total-serum and antigen-specific IgGs in a wide variety of chronic inflammatory and autoimmune diseases, such as RA, SLE, autoimmune vasculitis, active spondyloarthropathy, Crohn's disease and adult periodontal disease[13,25,26,30,32,42,43,50–53]. In support of the IgG–G0F glycoform as a biomarker, fluctuations in the abundance of IgG–G0F have been shown to correlate with the general presence of disease activity in patients with RA. Furthermore, levels of IgG galactosylation in patients with RA have been shown to increase upon treatment with methotrexate, or in women with RA during pregnancy, in which the levels of disease activity are generally reduced[54–56]. Data from the past 2 years suggest that increases in the abundance of G0F glycoforms might even occur shortly before the onset of disease in patients with RA and in those with PR3-anti-neutrophil cytoplasmic antibody (PR3–ANCA)-associated vasculitis, thus generating a time window for the initiation of a preventive treatment[41,42] (FIG. 3). Such increases in IgG–G0F glycoform levels have been detected in serum IgGs from patients with vasculitis. In RA, a similar increase in IgG–G0F glycoforms was described in those with anti-citrullinated antibodies[41,42]. An important question remains, however, as to whether these agalactosyl IgG glycoforms might be more than biomarkers and, in fact, have increased levels of pro-inflammatory activity, similar to that of IgGs lacking core fucose residues. Indeed, the findings of early studies involving mouse models of arthritis suggest that IgG–G0F autoantibodies might have an increased level of pro-inflammatory activity, owing to activation of the mannose-binding protein C (also known as mannose-binding lectin; MBL)-dependent complement pathway[57,58]. However, data from consecutive studies in MBL-deficient mice have demonstrated that transfer of serum from mice with

Box 1 | **IgG glycosylation and autoimmunity**

A long-standing connection exists between autoimmunity and antibody glycosylation. In many autoantibody-dependent and autoantibody-independent autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and inflammatory bowel disease, changes in antibody glycosylation were noted during active disease. Most commonly, the prevalence of the IgG–G0F glycoform, which lacks terminal galactose and sialic acid residues, is increased during inflammation[24–34]. Following the observation that terminally galactosylated and sialylated glycoforms might also have anti-inflammatory effects, a lack of these glycoforms was suggested not only to be a biomarker, but also a regulatory element in both the initiation and resolution of inflammation. This finding has raised great interest in the clinical potential of these IgG glycovariants as next-generation anti-inflammatory agents.

inflammatory arthritis, which is either enriched with IgG–G0F glycoforms or has been treated with galactosidase enzymes to generate pure IgG–G0F antibodies, is not dependent on the MBL signalling pathway and does not promote IgG-mediated inflammation[59,60]. Further evidence against a role of MBL as a determinant of IgG–G0F activity is provided by two studies involving patients with RA, demonstrating that polymorphisms in *MBL*, leading to increased or decreased MBL levels, do not correlate with disease activity[61,62]. Thus, the IgG–G0F glycoform is unlikely to have an increased level of pro-inflammatory activity compared with that of other IgG glycoforms. Convincing data are available, however, suggesting that highly galactosylated IgG glycoforms might have an anti-inflammatory effect if they are present as an immune complex[63]. In mice, the injection of galactosylated mouse IgG1 immune complexes interfered with the complement-dependent recruitment of neutrophils into the peritoneum and the skin. Of note, the inhibitory effect of highly galactosylated mouse IgG1 immune complexes on neutrophil migration was shown to be dependent on the association of the inhibitory FcγRIIb with dectin-1 (also known as C-type lectin domain family 7 member A), which blocked the transduction of activating signals via the C5a anaphylatoxin chemotactic receptor at the level of mitogen-activated protein kinase 1/3 phosphorylation[63]. The results of this study are consistent with data suggesting that galactosylation is an important determinant of the affinity of IgG1 for the FcγRII2b in mice[60]. More importantly, these results emphasize that future studies investigating the influence of IgG glycovariants on Fc-receptor binding should also investigate the role of immune complexes and not only that of monomeric IgG glycosylation variants.

Changes in antibody sialylation

N-Acetylneuraminic acid is found at the nonreducing end of *N*-glycans, distal to the asparagine residue that serves as the anchor in the polypeptide backbone (FIG. 1). In contrast to the abundance of highly sialylated sugar structures in other serum proteins or, in select examples, in which the IgG variable domain contains a glycosylation site, the Asn-297-linked sugar domain only contains a minor fraction of monosialylated and, very rarely, disialylated sugar structures[13]. Similar to results indicating a decrease in the prevalence of galactosylated IgG glycovariants during inflammation, several investigators have reported a correlation between the level of IgG sialylation and the extent of disease activity. For example, low levels of serum IgG or autoantibody sialylation have been observed in patients with RA, GPA, antiphospholipid syndrome (APS), vasculitis or SLE[10,34,41,64,65]. A reduction in the abundance of sialylated IgG glycovariants has been shown to precede disease relapse, and thus shows promise as a predictive biomarker of the need for early initiation of treatment[41,42] (FIG. 3). Moreover, in patients with Kawasaki disease and in those with Guillain–Barre syndrome, a treatment response is correlated with the restoration of serum autoantibody sialylation[66,67]. To understand how serum and autoantibody sialylation might affect antibody function and how this modification is relevant to autoimmune diseases, at least two aspects need to be considered: the effects of sialylation of the IgG Fc domain on modulating the interaction with downstream pro-inflammatory effector pathways (such as FcγR or complement activation); and the role of IgG Fc domain sialylation in generating active anti-inflammatory activity.

The findings of several independent studies have demonstrated that a high level of sialylation of sugar moieties in the IgG Fc domain might result in decreased IgG activity owing to a reduction in the affinity of mouse IgG1, IgG2b and human IgG1 antibodies for both the classical Fc receptor and complement effector pathways[68–70]. A possible explanation for this altered binding affinity for FcγRs would be that sialylation of IgG Fc sugar moieties leads to changes in IgG structure. Consistent with this scenario, the findings of some studies suggest that, similar to aglycosylated IgGs, the two C_H2 domains of highly sialylated IgG antibodies might be more prone to forming a closed conformation, which would impair FcγR binding[71,72]. The findings of other studies, however, reveal no effect of IgG sialylation on Fc structure[73,74]. Consistent with a minor effect on Fc structure, reports exist showing that specific human IgG1 antibodies, with high levels of Fc domain sialylation (and a reduced level of FcγR activation), were unaltered or only slightly reduced in their capacity to bind with activating FcγRs[70,74–76]. However, in one of these studies investigators observed an effect of sialylation on cognate antigen binding, suggesting that an altered level of hinge flexibility might cause this reduced affinity[70], again consistent with an effect of IgG sialylation on IgG structure. Also in line with such a hypothesis, researchers showed that the nephritogenic capacity of mouse IgG3 rheumatoid factor antibodies can be reduced by sialylation[77]. Thus, sialic-acid-containing glycovariants of rheumatoid factor antibodies have a strongly reduced propensity to become deposited in the kidneys and cause glomerulonephritis. In summary, the current evidence from the majority of independent *in vivo* studies suggests that sialylated IgG antibodies have a reduced

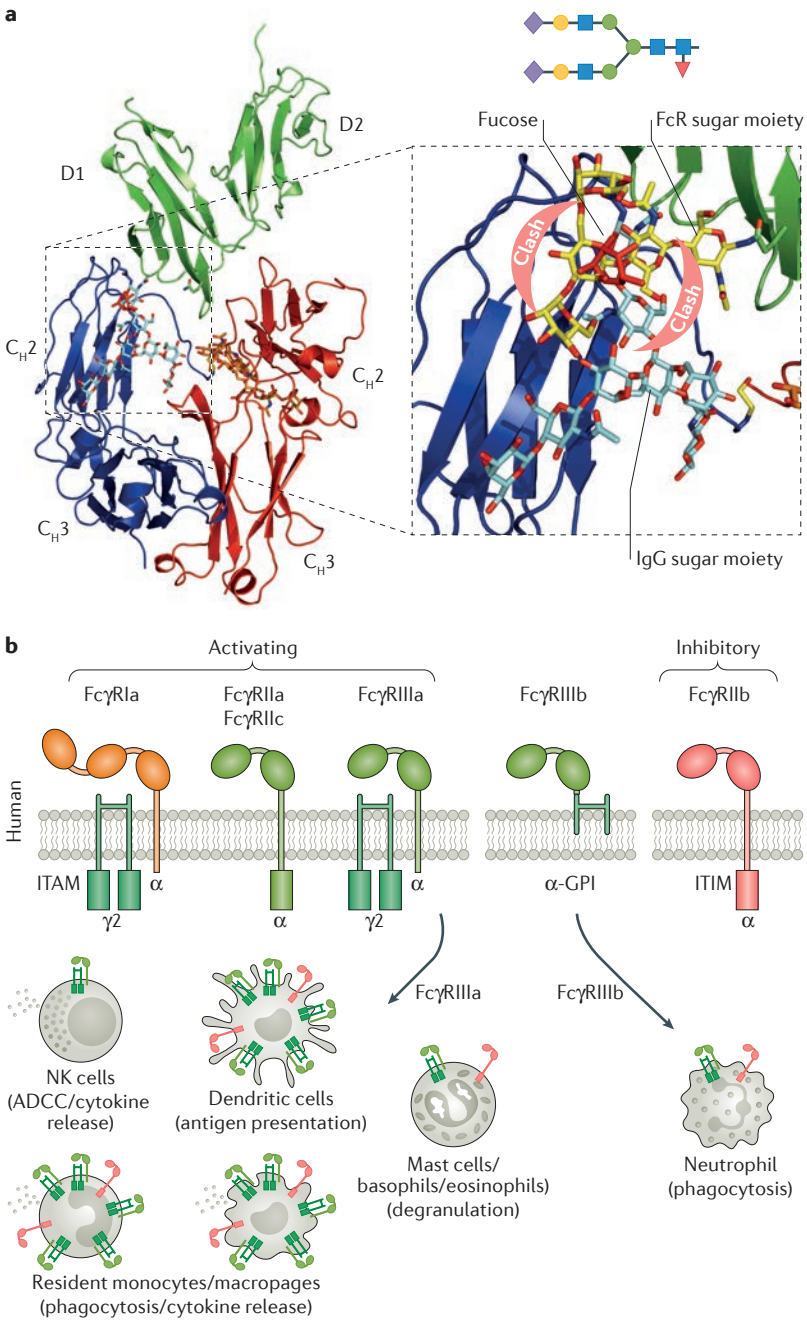

Figure 2 | **Influence of fucosylation on IgG effector functions. a** | Structure of the human low-affinity immunoglobulin-γ Fc region receptor III-A (FcγRIIIa, green) bound to the human IgG1 Fc domain (blue and red), with magnification of the interaction site of both sugar moieties of the FcγRIIIa (depicted as a yellow ball-and-stick model) and of one IgG Fc domain (blue ball-and-stick model). The fucose residue of the antibody sugar moiety (depicted in red) clashes with the sugar domain of FcγRIIIa, resulting in low-affinity binding. **b** | Activating (FcγRIa, IIa, IIc, IIIa) and inhibitory (FcγRIIb) FcγRs. These receptors mediate signalling through immunoreceptor-tyrosine-based activatory (ITAM) or inhibitory (ITIM) motifs. FcγRIIIb is linked via a glycerolphosphatidylinositol (GPI) anchor to the plasma membrane and does not connect with the activating FcRγ-chain. Green arrows indicate the increased selectivity of IgG glycosylation variants without fucose for FcγRIIIa (expressed on mast cells, basophils, eosinophils, dendritic cells, monocytes, macrophages, natural killer (NK) cells) and FcγRIIIb (expressed on neutrophils). Increased binding of IgGs to effector cells might result in an increase in magnitude of the depicted effector responses. ADCC, antibody-dependent cell-mediated cytotoxicity. Part **a** modified with permission from Nimmerjahn, F. & Ravetch, J. V. Fcgamma receptors as regulators of immune responses. *Nat. Rev. Immunol.* **8**, 34–47 (2008).

level of activity. With respect to the underlying mechanisms, however, antibody-intrinsic, Fc-dependent and Fab-dependent effects might both be involved. Additional research will be necessary to resolve this current debate. Interestingly, the negative effects of sialic acid on IgG activity (while having unchanged FcγR affinity[76]) can be overcome if fucose residues are not present in the sugar domain[75]. Highly sialylated, afucosylated IgG glycovariants are very rare, therefore, the relevance of this finding to naturally occurring autoantibodies remains to be determined. Moreover, an absence of fucose only enhances the affinity for FcγRIIIa, which would predict that FcγRIIa-dependent activities might still be impaired.

Coming back to the potential use of autoantibodies in the diagnosis of autoimmune diseases, a reduction of autoantibody-dependent effector functions mediated by sialic acid residues is consistent with IgG autoantibodies being present without causing disease. Evidence supporting this theory has been obtained by several groups during the past 3 years using different experimental models of inflammatory arthritis and in patients with RA or GPA. For example, immune complexes have been shown to be unable to induce osteoclastogenesis and bone loss *in vitro* and *in vivo* if they are highly sialylated[68,78]. In patients with active GPA, anti-PR3 antibodies were less sialylated compared with those obtained from patients without active disease, or from those with no history of autoimmune disease[10]. More importantly, the authors demonstrated that purified anti-PR3 antibodies from patients with active disease triggered a greater *in vitro* neutrophil-mediated oxidative burst than those from patients without active disease. Providing direct proof of the important role of autoantibody sialylation as a modulator of antibody activity, enzymatic desialylation of anti-PR3 antibodies from patients with inactive GPA restored pathogenic activity[10]. Furthermore, ACPA autoantibodies have been shown to develop a glycosylation pattern lacking in terminal sialic acid and galactose residues before the onset of, or during active arthritis[8,42,79]. In addition, sialylation of ACPA autoantibodies has been demonstrated to interfere with the development of collagen-induced arthritis in mice[79]. Conversely, deleting the B cell sialic acid transferase responsible for adding terminal sialic acid residues resulted in more-severe collagen-induced arthritis, following immunization with type II collagen. Finally, in a different study, mice deficient in IL-23 were protected from collagen-induced arthritis, despite the development of autoantibody responses comparable to those of mice susceptible to the induction of collagen-induced arthritis[80]. The passive transfer of active autoantibodies was able to induce arthritis in IL-23-deficient mice, and the effector pathways responsible for induction of inflammation seemed to be unaffected. Interestingly, analysis of the autoantibody glycosylation induced by immunization with type II collagen revealed that the presence of sialylated glycovariants did not decrease in IL-23-deficient mice[80]. The findings of more in-depth investigations demonstrated that IL-23 stimulates T helper 17 (T_H17) cells to secrete IL-21 and IL-22, which

were responsible for reducing the expression of the β-galactoside α-2,6-sialyltransferase 1 (REF. 80) (FIG. 3b). Providing direct evidence that IL-23 is a decisive factor in the downregulation of autoantibody sialylation in plasma cells, the authors also demonstrated that antibody-mediated neutralization of IL-23 in mice that spontaneously develop inflammatory arthritis was sufficient to decrease the extent of disease severity, owing to increased levels of antibody sialylation[80]. Consistent with this effect of T_H1 and/or T_H17 cytokines on IgG sialylation, findings from two previous studies using models of acute airway and kidney inflammation have demonstrated that immunizing mice with T cell-independent or T cell-dependent antigens without an adjuvant induces sialylation of antigen-specific antibodies[81,82]. More importantly, the transfer of sialylated antibodies has been demonstrated to ameliorate the severity of arthritis and acute airway inflammation in mice, suggesting that highly sialylated autoantibodies might not simply be impaired in their ability to recruit pro-inflammatory effector pathways but might also have an active immunomodulatory function and trigger resolution of inflammation[79,81] (FIG. 3a).

These findings are consistent with a body of literature demonstrating that sialylated IgG glycovariants might, at least in part, be responsible for the anti-inflammatory effects of polyclonal IgG preparations pooled from thousands of donors (intravenous immunoglobulin; IVIG therapy)[4,14] (BOX 2). Thus, research by several independent groups has demonstrated that depleting sialic-acid-rich glycosylation variants from an IVIG preparation abrogates the anti-inflammatory activity of such variants in a number of inbred and humanized mouse model systems, including models of nephrotoxic nephritis, ITP, epidermolysis bullosa acquisita, acute inflammatory airway hyperresponsiveness and autoantibody-mediated nerve injury[83–88]. By contrast, IVIGs enriched for Fc-domain sialylation, or recombinant sialylated human IgG1 Fc-fragments have an increased level of activity compared with other IVIGs, demonstrating that increasing levels of IgG sialylation in order to enhance the anti-inflammatory activity of IVIG therapy is possible[68,87,89–91].

Several molecular and cellular pathways of sialylated-IgG-dependent immune modulation that are specific to the autoimmune disease under investigation have been

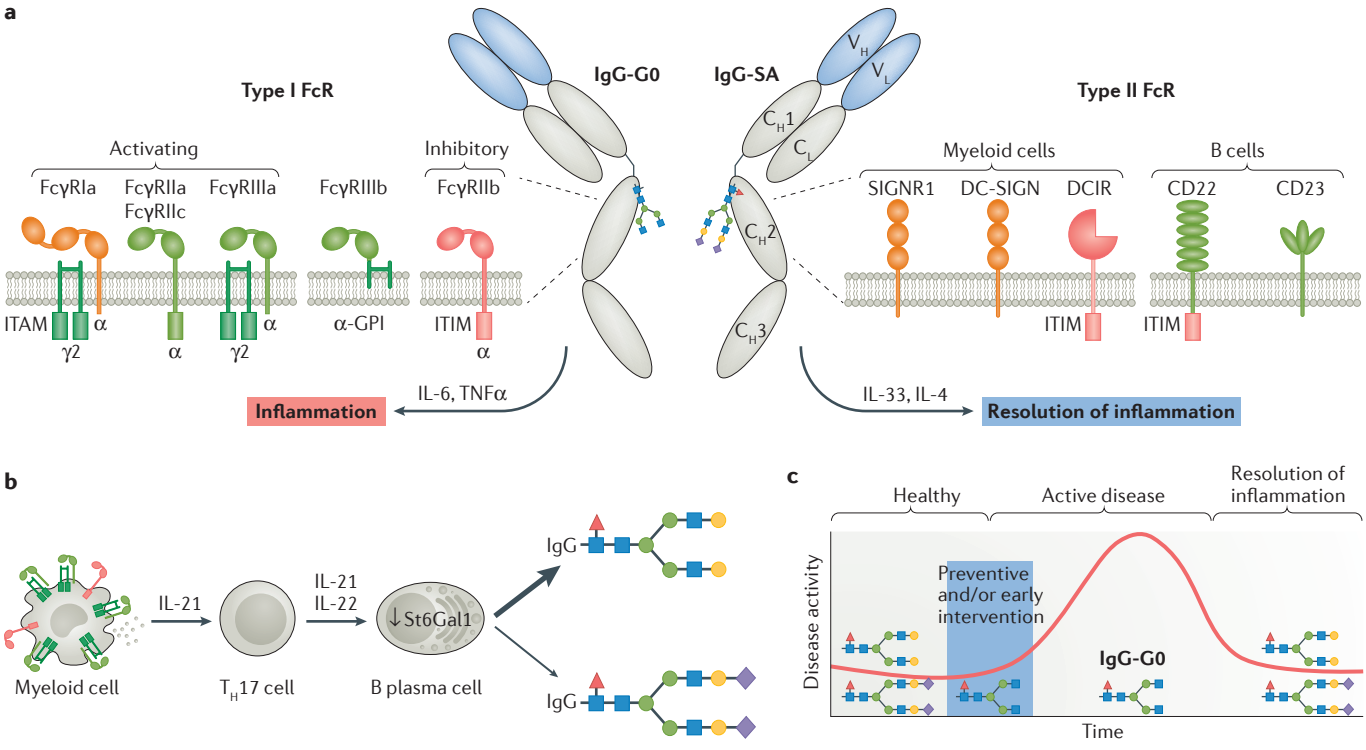

Figure 3 | **Effect of sialylated glycoforms on IgG activity. a** | Depending on the presence (IgG–SA) or absence (IgG–G0) of terminal sialic acid residues, IgG molecules can bind with either type I or type II Fc receptors (FcRs). Type I FcRs trigger innate immune-effector cell activation and pro-inflammatory cytokine release via activating and inhibitory FcγRs, whereas type II FcRs can initiate resolution of inflammation via the release of T helper 2 (T_H2) cytokines. **b** | Regulation of IgG sialylation. Upon IL-23 secretion by myeloid cells, T_H17 cells produce IL-21 and IL-22, which induces downregulation of the sialyltransferase β-galactoside α-2,6-sialyltransferase 1 (St6Gal1) in B cells. Lack of St6Gal1 expression results in the generation of IgG glycovariants deficient in terminal sialic acid residues. **c** | Schematic representation of changes in IgG glycosylation during the remitting phase of an autoimmune disease, such as rheumatoid arthritis. Galactosylated and sialylated IgG glycovariants are present before, or following resolution of disease, whereas an increase in the prevalence of IgG–G0 glycovariants might appear shortly before disease initiation, thus generating a time window for use of a preventive or early intervention using anti-inflammatory medications, such as intravenous immunoglobulin (IVIG) infusion, cytokine neutralization, or glucocorticosteriods. α-GPI, glycerolphosphatidylinositol; DCIR, C-type lectin domain family 4 member A; DC-SIGN, CD209 antigen; ITAM, immunoreceptor-tyrosine-based activatory motif; ITIM, immunoreceptor-tyrosine-based inhibitory motif; SIGNR1, CD209 antigen-like protein B.

Box 2 | **The use of IVIG in autoimmunity**

The use of pooled human serum IgGs in the treatment of chronic inflammatory and autoimmune diseases (intravenous immunoglobulin; IVIG therapy) dates back to the 1980s when it was noticed that the infusion of high doses (1–3 g/kg body weight) of serum IgG preparations ameliorated immunothrombocytopenia in children[126]. Following the original use of such infusions, IVIG has been used in a wide variety of autoimmune diseases, including immune thrombocytopenia, Kawasaki disease, skin-blistering diseases and chronic inflammatory demyelinating polyneuropathy. A unifying mechanism explaining the immunomodulatory effects of IVIGs for all different types of autoimmune diseases is still lacking, although the IgG Fc domain has been identified as a key element of the immunomodulatory activity. Furthermore, several preclinical model systems have provided evidence of this important role of sialylated IgG glycovariants in determining the therapeutic activity of IVIG[68,84–86,88,89,91,92,94]. The available data suggest that triggering resolution of inflammation by replenishing sialic-acid-rich IgG glycovariants would be one critical factor in triggering resolution of inflammation in patients with active autoimmune disease.

elucidated. Thus, mice deficient in the probable pathogen recognition receptor CD209 antigen-like protein B (CD209B, also known as SIGNR1) were no longer protected by IVIG in mouse models of inflammatory arthritis, ITP, or experimental autoimmune encephalomyelitis (EAE)[90–93]. Replacing CD209B with its human orthologue, CD209 antigen (also known as DC-SIGN), restored IVIG-dependent immunomodulatory activity in these model systems[90–92]. Apart from SIGNR1 and DC-SIGN, other C-type lectin receptors, including C-type lectin domain family 4 member A (CLEC4A, also known as DCIR) and B-cell receptor CD22 (CD22), were shown to be critically involved in IVIG-mediated amelioration of acute airway hyperresponsiveness[84] and inhibition of B cell responses, respectively[94] (FIG. 3A). How these type II FcRs contribute to sialylated IgG activity is currently an active area of investigation. The findings of various studies suggest that direct binding of sialylated IgG glycovariants can occur[72,90,92], although others suggest that this might not be the case[95]. Of note, research has also revealed that the requirement of specific receptors can differ depending on when treatment is initiated. For example, SIGNR1 seems to be more critical for IVIG activity in animals pretreated with IVIG, compared with those with established disease[86]. In summary, more research is necessary to fully elucidate the molecular and cellular pathways through which the effects of sialylated IgG glycovariants are mediated.

Apart from cell-surface receptors, studies involving mouse models of inflammatory arthritis and nerve injury mediated by anti-ganglioside antibodies suggest that sialylated IgG triggers the release of IL-33, which stimulates IL-4 release by basophils. IL-4 has been shown to upregulate expression of the inhibitory FcγRIIb on innate immune effector cells, thereby increasing the threshold for activation by autoantibody immune complexes[14,88,90]. Consistent with this observation in mice, a release of IL-33 was noted in patients with various inflammatory myopathies after IVIG infusion, although expansion of the basophil population was noted only in certain patients[96]. IL-33 and IL-4 are not essential for IVIG activity in animal models of noninflammatory autoimmune diseases such as ITP[86,93,97]. Moreover, while a broad consensus exists on the critical role of the IgG Fc domain for the immunomodulatory activities of IVIG, various studies have demonstrated that sialic-acid-rich glycoforms might not be required for IVIG activity in certain *in vivo* model systems[98–100]. More research will be necessary to identify the conditions under which sialic-acid-dependent and independent pathways are responsible for IVIG activity in these different mouse models. In 2015, a humanized mouse model system of ITP was developed using immunodeficient mice reconstituted with a human immune system[85]. These mice additionally lack the mouse forms of activating FcγRs, therefore, the authors could selectively study the effects of IVIG-mediated inhibition of platelet depletion using human autoantibodies and human effector cells *in vivo*. In line with observations in some inbred mouse-model systems, IVIG activity was critically dependent on sialic-acid-containing IgG glycovariants[85].

In addition to these passive models of autoimmunity, data from various studies have begun to address how IVIG modulates active autoimmune diseases such as experimental autoimmune encephalitis, where T cells, not autoantibodies, are critical for tissue pathology[91]. In an EAE model system, IVIG-mediated suppression of active disease was dependent on sialic-acid-rich glycovariants, which were shown to be responsible for induction of regulatory T cells through IL-33 signalling. Finally, another study exploring the activity of sialylated antibodies generated during an immune response in a mouse model of allergic airway responses demonstrated that sialylated antibodies were responsible for limiting the extent of airway inflammation[81]. In summary, the extent of IgG glycovariant sialylation is reduced in patients with many active autoimmune diseases, and the findings of several studies have demonstrated that this results in a reduction in the level of pro-inflammatory activity. This observation might, at least in part, explain why autoantibodies can be present in patients without disease. Moreover, detection of changes in autoantibody sialylation and/or galactosylation early in the course of disease, or even before the emergence of symptoms could hold some promise as a predictive biomarker of disease development.

Differential F(ab) glycosylation

Apart from the well-documented changes in IgG Fc glycosylation during inflammation, accumulating evidence indicates that sugar structures attached to the antibody variable domain (Fab-fragment) might also have a role in modulating autoantibody activity[101]. Depending on the method used for Fab glycosylation analysis, approximately one fifth of serum IgG molecules are estimated to contain *N*-linked sugar structures in their variable region[102,103]. The consensus sequence for *N*-linked glycosylation is not encoded in the majority of antibody germline sequences, such sequences have to be generated during the process of somatic hypermutation and can be found in both framework and complementarity-determining regions of antibody heavy and light chains[104,105]. All *N*-linked glycosylation sites in the Fc domain are occupied by a sugar moiety, however glycosylation sites in the Fab domain might

not always become glycosylated. Moreover, depending on where the glycosylation site is introduced into the variable region, the sugar moiety can either be of a complex-biantennary or of a high-mannose type[106,107]. Sugar moieties of the biantennary type can be highly processed, with terminal sialic acid residues typically detected in 80–90% of such moieties[52,108] and, the presence or absence of sialylation can affect antibody half-life. F(ab) glycosylation has been found to either enhance or prevent antigen binding[109–111]. Elegant research involving mouse model systems has demonstrated that F(ab) glycosylation can prevent potentially autoreactive antibodies from becoming autoreactive by lowering their affinity for self-antigens[112]. Although changes in F(ab) glycosylation have been observed in patients with RA, the presence of such changes has not been shown to correlate with disease development or with the severity, or resolution of inflammation[113,114]. Interestingly, ACPAs in patients with RA have been demonstrated to be abundantly glycosylated in the Fab region, which might either increase or decrease the extent of antigen affinity[115]. This high level of complexity, and the opposing outcomes associated with different patterns of Fab glycosylation, require a case-by-case evaluation of the specificity of each antibody under investigation and makes it difficult to draw general conclusions. Nonetheless, evidence exists that, similar to the critical role of sialic acid residues in the Fc-associated sugar moiety, terminal sialic acid residues in the Fab-associated sugar moiety might have anti-inflammatory activity owing to inhibition of production of pro-inflammatory cytokines and chemokines such as IFNα or monocyte chemotactic protein 1 (MCP1; also known as CCL2), at least *in vitro*[116,117]. However, the findings of three clinical trials[118–120] have demonstrated that the infusion of IVIG Fc fragments is sufficient to produce a therapeutic effect in children and adults with ITP or Kawasaki disease. Further research, involving suitable *in vivo* model systems or clinical trials, will be critical to establishing the relevance of these observations to human autoimmune diseases. Moreover, while changes in Fab glycosylation have been demonstrated to occur during pregnancy in women with arthritis, these features are not associated with the severity of the disease[108,113]. In summary, an interesting correlation exists between Fab glycosylation and certain autoantibody species, such as ACPAs, which might provide insights into how these autoantibody responses develop and how Fab glycosylation modulates autoantibody specificity.

Conclusions

Following the first description that changes in serum IgG glycosylation occur in patients with autoimmune diseases >40 years ago, great progress has been made in our understanding of how IgG glycosylation affects (auto)antibody function. IgG glycoforms lacking in terminal sialic acid and galactose residues not only correlate with the initiation and resolution of inflammation, but might precede disease initiation, and hence, could be used as biomarkers to guide the use of preventive treatments in patients (FIG. 3c). This approach might enable the onset of tissue pathology to be prevented, and, in the long run, might even enable complete prevention of disease flares. Using our knowledge of the anti-inflammatory and immunomodulatory activities of highly galactosylated immune complexes and sialylated monomeric IgG molecules might enable new treatment avenues for the induction of rapid resolution of inflammation to be explored, and thus prevent the onset of tissue damage. In addition, more knowledge of factors that can change antibody glycosylation *in vivo* might help to maintain autoantibodies in an inactive state. We are beginning to define the circumstances in which differentially glycosylated IgG variants appear *in vivo*, and the genetic factors that influence their generation[121]; however, we do not fully understand the molecular and cellular pathways responsible for these changes. In this respect, the identification of IL-23 as a modulator of sialyltransferase expression in B cells *in vivo* has been a major advance in our understanding[80]. Identifying further key molecules that influence autoantibody glycosylation and turn pro-inflammatory antibodies into inhibitors of disease pathology in humans would be a further major breakthrough. Understanding why certain alloantigens trigger afucosylated antibodies, for example, could finally uncover how these inhibitory IgG glycovariants can be generated through immunization. However, the demonstration that afucosylated virus-specific IgG glycovariants might explain the antibody-dependent enhancement of dengue virus infection highlights that the immune system must be able to distinguish between the need to allow an optimal level of effector-cell recruitment via the IgG Fc-domain, and possible negative effects, such as excessive activation of the immune system, with enhancement of virus-induced pathology[122]. Thus, the stability of IgG fucosylation during inflammatory processes might be a checkpoint to monitor for the prevention of tissue damage. Finally, understanding how differential glycosylation of other immunoglobulin isotypes, such as IgA and IgE, affects their activity will be of great interest. For example, the occurrence of aberrant galactosylation of IgA1 in patients with IgA nephropathies has long been established and this feature might be a biomarker that enables the prediction of disease development[123,124]. Furthermore, glycosylation was shown, as recently as 2015, to be critical for IgE activity[125]. Both, IgA and IgE contain multiple complex sugar moieties, therefore, identifying the effects of these different domains and individual sugar residues on antibody function will be very challenging. Nonetheless, specific sugar moieties on IgEs have been shown to be critical for the activity of IgEs *in vivo*[125]. Given that many of the changes in IgG glycosylation have been established for nearly 40 years, a question remains as to why this knowledge has not been incorporated into general clinical practice. Two exemplary issues include the very sophisticated and cost-intensive nature of glycoanalysis and the potential need to purify autoantibodies from patient serum. Once specific changes in autoantibody glycosylation have become firmly established for different autoimmune diseases, the use of simpler, lectin-based assays might enable the more-widespread use of altered (auto)antibody glycosylation as a diagnostic tool.

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Synovial tissue research: a state-of-the-art review

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In the original version of this article the name of one of the authors, Elsa Vieira-Sousa, was incorrectly given as Elsa Sousa. This error has now been corrected in the PDF and HTML versions of the article.