Nature Reviews Rheumatology | Published online 20 Oct 2017

IN BRIEF

AUTOINFLAMMATION

Stem cell transplantation for DADA2

Haematopoietic stem cell transplantation (HSCT) was an effective and definitive treatment in 14 patients with deficiency of adenosine deaminase 2 (DADA2), a monogenic autoinflammatory vasculopathy. All patients reported resolution of immunological and haematological phenotype, with no new vascular events at an average of 18 months follow-up, and adenosine deaminase 2 activity normalized as early as 14 days post-HSCT in those patients tested ($n = 7$). Adverse events were reported in 11 of the 14 patients (four incidents of cytopenia and seven incidents of moderate or acute graft-versus-host disease).

ORIGINAL ARTICLE Hashem H. *et al.* Hematopoietic stem cell transplantation rescues the hematological, immunological and vascular phenotype in DADA2. *Blood* http://dx.doi.org/10.1182/blood-2017-07-798660 (2017)

CRYSTAL ARTHRITIS

Febuxostat reduces synovitis in early gout

Compared with placebo, urate-lowering therapy with once-daily febuxostat at 40mg (or 80mg if serum uric acid was ≥6 mg/dL after 14 days) in patients with early gout (hyperuricaemia and ≤2 gout flares; $n = 183$) reduced MRI-detected synovitis (change from baseline RAMRIS score of −0.43 versus −0.07; $P < 0.001$), decreased the incidence of disease flares (29.3% versus 41.4%; $P < 0.05$) and increased the proportion of patients obtaining a serum uric acid level of <6 mg/dL (62.8% versus 5.7%; $P < 0.001$) after 24 months. However, treatment with febuxostat did not cause detectable changes in joint erosion over 2 years.

ORIGINAL ARTICLE Dalbeth, N. *et al.* Effects of febuxostat in early gout: a randomized, double-blind, placebo-controlled study. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40233 (2017)

PAEDIATRIC RHEUMATOLOGY

DNA methylation in oligoarticular JIA

Results from the analysis of DNA methylation patterns using Illumina HumanMethylation450 arrays showed no substantial differences between $CD4^+$ T cells from 56 patients with oligoarticular juvenile idiopathic arthritis (JIA) and $CD4^+$ T cells from 57 age-matched healthy individuals. The authors of the study suggest that this lack of a difference could indicate a less crucial role for epigenetic changes in JIA than in rheumatoid arthritis in adults.

ORIGINAL ARTICLE Chavez-Valencia, R. A. *et al.* The DNA methylation landscape of $CD4^+$ T cells in oligoarticular juvenile idiopathic arthritis. *J. Autoimmun.* http://dx.doi.org/10.1016/j.jaut.2017.09.010 (2017)

VASCULITIS SYNDROMES

Rituximab for adult-onset IgA vasculitis

In a multicentre observational study of 22 patients with adult-onset IgA vasculitis, 90.9% ($n = 20$) achieved remission (as defined by Birmingham Vasculitis Activity Score; BVAS) at an average follow-up of 24 months when receiving rituximab either as monotherapy or as an additional therapy. Following the initiation of rituximab, patients experienced reductions in BVAS ($P < 0.0001$) and levels of proteinuria ($P < 0.0001$) and C-reactive protein ($P = 0.0005$), and were able to reduce their dose of prednisone. Of those patients who achieved remission, 35% ($n = 7$) subsequently experienced a relapse.

ORIGINAL ARTICLE Maritati, F. *et al.* Rituximab for the treatment of adult-onset IgA vasculitis (Henoch-Schönlein purpura). *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40339 (2017)

Nature Reviews Rheumatology | Published online 21 Nov 2017; doi:10.1038/nrrheum.2017.190

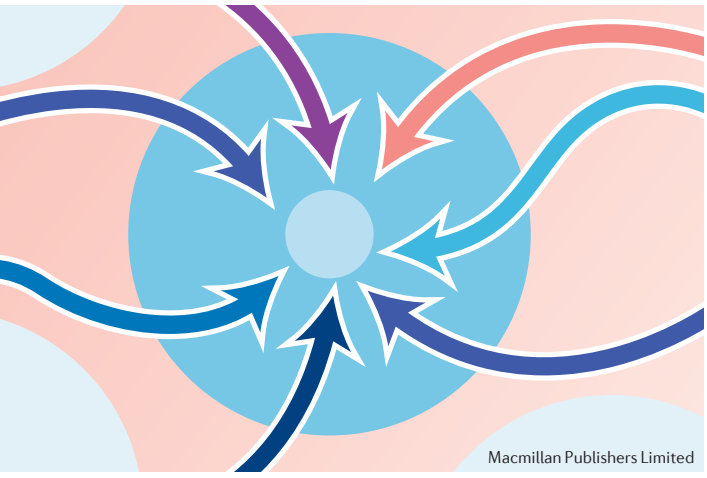
SYSTEMIC SCLEROSIS

STAT3 — A key integrator of profibrotic signalling

Signal transducer and activator of transcription 3 (STAT3) could be a prime target for treating fibrosis in diseases such as systemic sclerosis (SSc), according to new findings published in *Nature Communications*. Multiple kinases, including those downstream of transforming growth factor-β (TGFβ) and IL-6, are thought to contribute to the aberrant activation of fibroblasts observed in fibrotic diseases. "We provide the first evidence that STAT3 serves as a key molecular checkpoint in fibroblasts by integrating and converting signals from these kinases into profibrotic responses," reports Debomita Chakraborty, first author of the study.

The molecular mechanisms that govern aberrant fibroblast activation in fibrosis are incompletely understood; although studies have identified TGFβ as a key molecule in this process, inhibition of individual pathways downstream of TGFβ do not completely abrogate its profibrotic effects. STAT3, a transcription factor activated downstream of TGFβ, is constitutively expressed in many tissues and cells where it is influenced by various stimuli and has been implicated in the pathogenesis of several diseases. Furthermore, a number of strategies for targeting STAT3 are currently being tested in clinical trials. "These various aspects led us to analyse the activation of STAT3 in SSc and also investigate its role in TGFβ-dependent fibroblast activation and tissue fibrosis," comments corresponding author Jörg Distler.

> "a number of strategies for targeting STAT3 are currently being tested in clinical trials"



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The researchers observed an increase in the activated (phosphorylated) form of STAT3 (pSTAT3) in tissue from skin biopsies and cultured fibroblasts from patients with SSc compared with age-matched and sex-matched healthy individuals. Recombinant TGFβ induced the phosphorylation of STAT3 in cultured human fibroblasts, which was associated with the stimulation of several kinases; individual inhibition of these kinases reduced TGFβ-induced phosphorylation of STAT3. Similarly, selective pharmacological inhibition of either TGFβ or these individual kinases reduced bleomycin-induced phosphorylation of STAT3 in mice.

Blocking STAT3 signalling with a small molecule inhibitor (S31-201) inhibited TGFβ-induced fibroblast-to-myofibroblast transition and collagen release in cultured human fibroblasts; similar findings were observed with genetic deletion of *Stat3* in mouse fibroblasts, suggesting that the effects of S31-201 were direct and not due to off-target activity.

"Targeting STAT3, downstream of both … IL-6 and TGFβ, would be predicted to be effective in SSc as you are blocking the convergence point," comments Steven O'Reilly, who was not involved with this study. "We have previously demonstrated the importance of IL-6-mediated activation of STAT3 in fibrosis generation," he explains. "Thus blocking STAT3 may block the effects of both cytokines in the disease."

To investigate STAT3 targeting *in vivo,* Chakraborty *et al.* used two mouse models of fibrosis: bleomycin-induced fibrosis (a model of early, inflammation-driven fibrosis) and fibrosis induced by the overexpression of constitutively active TGFβ receptor type I (a model of late-stage, non-inflammatory SSc associated with endogenous activation of fibroblasts). Inhibition of STAT3 by either S31-201 or fibroblast-specific genetic inactivation ameliorated fibrosis in both models. "These findings provide proof that STAT3 directly regulates fibroblast activation independently of its well-known effects on inflammation," states Chakraborty.

"The demonstration of direct, inflammation-independent effects on fibroblasts is of high translational relevance given that only one-third of patients with SSc show clinical evidence of inflammation," comments Distler. As SSc is a heterogeneous disease, with variation in the individual upstream profibrotic pathways that are activated, they propose that the identification of such a central signalling nexus could ensure therapeutic effects across different subpopulations of patients.

"We thus demonstrate that STAT3 is a central integrator of multiple profibrotic signals and a promising candidate for molecular targeted therapies of fibrosis in SSc," concludes Chakraborty.

Jessica McHugh

ORIGINAL ARTICLE Chakraborty, D. *et al.* Activation of STAT3 integrates common profibrotic pathways to promote fibroblast activation and tissue fibrosis. *Nat. Commun.* **8**, 1130 (2017)

Nature Reviews Rheumatology | Published online 21 Nov 2017; doi:10.1038/nrrheum.2017.192

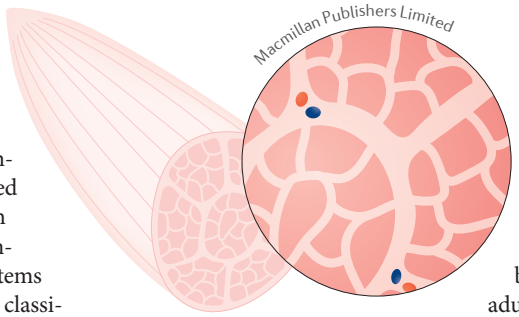
INFLAMMATORY MYOPATHIES

New classification criteria developed for research

> "the most useful feature of the classification system is its in-built flexibility"

The International Myositis Assessment and Clinical Studies (IMACS) network was established in the late 1990s to develop assessment tools for myositis disease activity. In the course of that work, the need became apparent for reliable classification criteria that were data driven, useful for clinical trial enrolment and research purposes, and were inclusive of adult and paediatric patients. Now, the IMACS-led International Myositis Classification Criteria Project (IMCCP) presents new guidelines that are supported by the ACR and EULAR.

The IMCCP comprised experts from several specialties who treat myositis, including rheumatology, dermatology, neurology and paediatrics. Together, these experts designed the study and validation experiments, revised the list of criteria for assessment and designated the inclusion criteria for cases (a diagnosis of known confidence for at least 6 months before inclusion) and comparators (individuals without myositis). In totaly, 976 patients were enrolled from 47 centres in Europe, the United States and Asia, of which 74% were adult and 26% were children; 624 comparators were also included. In looking at different classification techniques, a probability-score model was deemed to have superior discriminating performance compared with sum-of-items and classification-tree approaches and was taken forward and refined.



"The probability-score model gave the best sensitivity and specificity to distinguish myositis based on 16 variables, each given a specific weighted score," explains corresponding author Ingrid Lundberg. The variables include age of onset, type and location of muscle weakness, skin manifestations, laboratory findings and muscle biopsy findings. "A sum of the score gives a probability of having myositis, where the cut-off score of 55% indicates 'probable' myositis," Lundberg continues. For those individuals who reach a score of ≥90%, the classification is deemed 'definite'; patients in the probability score range of 50–55% are classed as having 'possible' myositis.

Arguably, the most useful feature of the classification system is its in-built flexibility: not all variables need to be assessed to reach a classification of myositis. As the criteria encompass patients with all the major subtypes of myositis — dermatomyositis, polymyositis and inclusion body myositis in adults, and juvenile dermatomyositis in children — those with, for example, heliotrope or Gottron rashes can be classified as having dermatomyositis without undergoing muscle biopsy. Additionally, the research network developed a simple classification tree to help researchers determine the subtype of myositis.

Going forward, the classification score will need to be validated in independent cohorts of patients and comparators. "We will also have to update the criteria when we have access to cohorts who have been tested for emerging myositis-specific autoantibodies," explains Lundberg, as the current classification only includes anti-Jo1 autoantibodies.

Mina Razzak, Chief Editor,
Nature Reviews Disease Primers

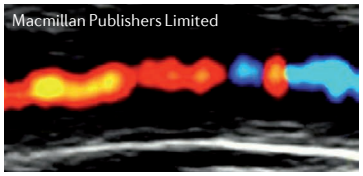
ORIGINAL ARTICLE Lundberg, I. E. *et al.* 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40320 (2017)

Nature Reviews Rheumatology | Published online 3 Nov 2017; doi:10.1038/nrrheum.2017.184

VASCULITIS SYNDROMES

Ultrasonography in GCA assessment

Although colour duplex ultrasonography (CDS) is increasingly accepted as a diagnostic tool for giant cell arteritis (GCA), this imaging modality is still not widely used in routine clinical practice, owing largely to the need for skilled sonographers. A retrospective analysis of CDS findings in 293 patients with suspected or confirmed large-vessel vasculitis suggests CDS could reduce the need for temporal artery biopsy (TAB) — currently the gold standard for diagnosing GCA, but an invasive procedure — and also provides support for the role of CDS in the detection of disease flares in follow-up.



Monti and colleagues used a CDS protocol based on previous evidence and initially developed and tested as part of the Temporal Artery Biopsy vs Ultrasound in Diagnosis of GCA (TABUL) study, with standardized methods of sonographer training and interpretation of CDS findings. The 293 consecutive patients referred to a fast-track outpatient clinic between July 2014 and September 2016 were assessed clinically by a rheumatologist and underwent CDS of the temporal arteries and axillary arteries at each visit.

Among the 210 patients with suspected GCA, a diagnosis was clinically confirmed in 118. CDS findings were positive in 52 of these 118 (44%) cases. Notably, CDS had a sensitivity of 63.3% and a specificity of 100% in new referrals treated with glucocorticoids for <7 days; among those treated with glucocorticoids for >7 days, the specificity remained high (98.3%) but sensitivity dropped to 43.6%. In patients with jaw claudication and high inflammatory markers, sensitivity rose to 81.8%.

The rate of TAB declined over the observation period, from 72 (42%) in 2014–2015 to 36 (25%) in 2016 ($P = 0.002$), which the authors suggest reflected increased recognition that positive CDS provided sufficient evidence of GCA. CDS was also able to detect changes in 'halo' distribution during follow-up and could be useful in detecting disease flares.

Sarah Onuora

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FURTHER READING Dejaco, C. *et al.* Giant cell arteritis and polymyalgia rheumatica: current challenges and opportunities. *Nat. Rev. Rheumatol.* **13**, 578–592 (2017)

> "…[colour duplex sonography] could reduce the need for temporal artery biopsy…"

Nature Reviews Rheumatology | Published online 26 Oct 2017; doi:10.1038/nrrheum.2017.180

INFLAMMATION

Specific inhibition of NLRP3 comes closer

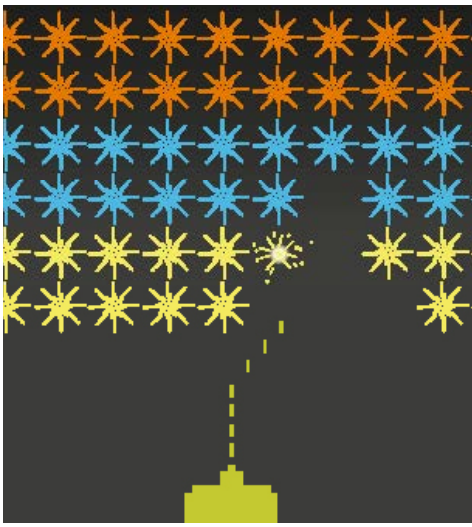
> CY-09 prevented neonatal death in a mouse model of Muckle–Wells syndrome (a cryopyrin-associated periodic syndrome)

The past few years have seen an explosion of interest in the role of the NOD, LRR and pyrin domain-containing 3 (NLRP3) inflammasome in both autoinflammatory diseases and rheumatic diseases. Several inhibitors of the NLRP3 inflammasome are currently being developed as potential therapeutics, but some compounds have unwanted off-target effects. Now, the latest report in a long line of studies showcases a small-molecule inhibitor that is highly specific for NLRP3.

"Although both the components of the NLRP3 inflammasome and the related signalling events can be targeted to inhibit NLRP3 inflammasome activation, only directly targeting NLRP3 itself can specifically inhibit the NLRP3 inflammasome," states corresponding author Rongbin Zhou. "To look for a direct NLRP3 inhibitor, we first screened compound libraries, then excluded the compounds that can inhibit NF-κB or the activation of other inflammasomes, or that have effects on the upstream signalling events of NLRP3, including mitochondrial damage, potassium efflux and chloride efflux."

Zhou and colleagues found that the resulting molecule, CY-09, specifically bound to the ATP-binding motif of NLRP3 and inhibited its ATPase activity, blocking assembly of the NLRP3 inflammasome. *In vivo*, CY-09 prevented neonatal death in a mouse model of Muckle–Wells syndrome (a cryopyrin-associated periodic syndrome) and reduced insulin insensitivity in mice fed a high-fat diet (a model of type 2 diabetes mellitus). CY-09 also inhibited caspase-1 activation and IL-1β production *ex vivo* in cells isolated from the synovial fluid of patients with gout.

"At this point in the field of NLRP3 inhibitors, the next question is whether the concentrations, which are effective *in vitro* and are used in mice, are safe and effective in humans," comments Charles Dinarello, who was not involved in the study. "Specific NLRP3 inhibitors have the potential to treat acute diseases such as gout flares, but also, more importantly, chronic diseases such as heart failure, atherosclerosis and Alzheimer disease; however, CY-09 is a long way off from clinical reality," he concludes.



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Joanna Collison

ORIGINAL ARTICLE Jiang, H. *et al.* Identification of a selective and direct NLRP3 inhibitor to treat inflammatory disorders. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20171419 (2017)

Nature Reviews Rheumatology | Published online 9 Nov 2017; doi:10.1038/nrrheum.2017.189

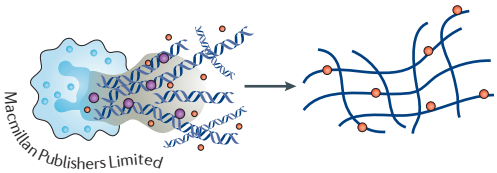
VASCULITIS SYNDROMES

NET production complements endothelial damage

> NETs might act as a scaffold during the activation of complement proteins

The production of neutrophil extracellular traps (NETs) and subsequent endothelial dysfunction have previously been implicated in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), but the mechanisms involved in these processes have been unclear. New research has now revealed clear links between ANCA-induced neutrophil activation, NET formation, the alternative complement pathway and endothelial cell damage in murine models of AAV and in patients with AAV who have necrotizing and crescentic glomerulonephritis (NCGN).

NETs are produced by neutrophils as a form of controlled cell death. Necroptosis is the best-characterized form of regulated necrosis and is dependent on receptor-interacting serine/threonine protein kinase 1 (RIPK1) and RIPK3. Blocking RIPK1 in human neutrophils *in vitro* greatly reduced NET production upon stimulation with ANCAs, a result that was mirrored in neutrophils from mice deficient for RIPK3.



Having established that necroptosis is the pathway necessary for ANCA-stimulated NET production, the researchers proceeded to identify how NETs contribute to the endothelial cell damage that is typically seen in AAV. NETs isolated from ANCA-stimulated neutrophils were able to damage endothelial monolayers *in vitro*: this effect was prevented by either inhibition of necroptosis or degradation of NETs. Curiously, the researchers also noted that NETs produced in response to ANCAs contained complement proteins C5a and C3d. The alternative complement pathway is thought to be involved in endothelial cell damage, and an oral C5a inhibitor is currently under investigation for use in patients with AAV. The authors suggest that NETs might act as a scaffold during the activation of complement proteins.

In a passive transfer mouse model of AAV, treatment with DNase I to degrade NETs prevented the mice from developing disease, and in RIPK3-deficient mice, the same passive transfer model of disease was unable to produce the typical NCGN pathology. Examination of kidney tissue from patients with AAV and NCGN revealed specific staining for phoshphorylated mixed lineage kinase domain-like protein (a protein crucial for necroptosis) in glomerular neutrophils, suggesting that the necroptosis pathway is active in neutrophils in sites of active disease.

"Specific inhibitors of the necroptosis pathway are currently under development or are already being tested in phase II studies," explains corresponding author Adrian Schreiber. "Therefore, necroptosis inhibition could be a potential novel treatment approach for AAV."

Joanna Collison

ORIGINAL ARTICLE Schreiber, A. *et al.* Necroptosis controls NET generation and mediates complement activation, endothelial damage, and autoimmune vasculitis. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1708247114 (2017)

Nature Reviews Rheumatology | Published online 12 Oct 2017; doi:10.1038/nrrheum.2017.174

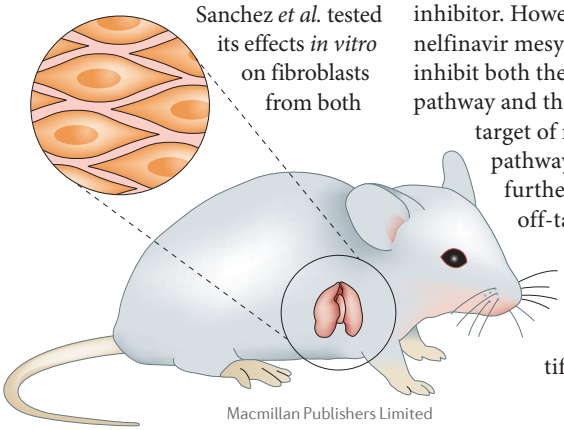
SYSTEMIC SCLEROSIS

Antiviral drug inhibits lung fibrosis

> Nelfinavir mesylate inhibited the development of lung fibrosis in an animal model of SSc

Nelfinavir mesylate, an antiretroviral drug used for the treatment of HIV, could be a potential new therapy for the treatment of systemic sclerosis (SSc) according to new findings published in *Arthritis & Rheumatology*. Nelfinavir mesylate inhibited the development of lung fibrosis in an animal model of SSc.

New therapies for SSc that have a lower toxicity and higher efficacy than currently used drugs are needed for treating pulmonary fibrosis. Nelfinavir mesylate is a safe approved drug for treating HIV. To investigate whether this drug can be repurposed for the treatment of SSc, Sanchez *et al.* tested its effects *in vitro* on fibroblasts from both healthy individuals and patients with SSc. Nelfinavir mesylate inhibited transforming growth factor β1 (TGFβ1)-mediated myofibroblast differentiation of lung, skin and ventricular fibroblasts in a dose-dependent manner, as demonstrated by a reduction in the expression of collagen, fibronectin and α-smooth muscle actin. Treatment with this drug also increased autophagic degradation of type I collagen by differentiating fibroblasts, resulting in reduced myofibroblast contractility.



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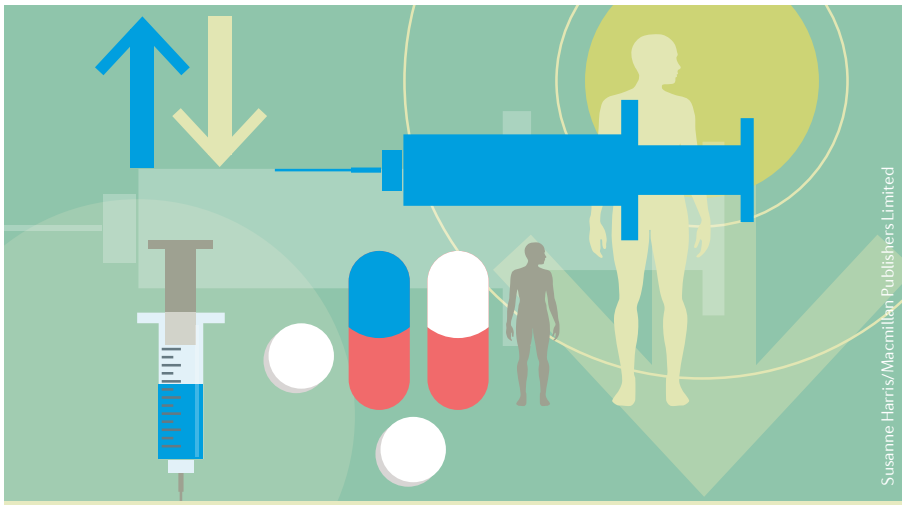
Mechanistically, nelfinavir mesylate inhibits HIV replication via its function as a protease inhibitor. However, in this study, nelfinavir mesylate was shown to inhibit both the canonical TGFβ1 pathway and the mechanistic target of rapamycin (mTOR) pathway in fibroblasts. To further investigate the off-target effects of this drug, Sanchez *et al.* used *in silico* proteome-wide screening to identify such interactions. TGFβ receptor 1 was identified as a top-scoring predicted target of nelfinavir mesylate, with results suggesting that nelfinavir mesylate has an inhibitory action on this receptor.

In a bleomycin-induced animal model of SSc, pretreatment with nelfinavir mesylate inhibited the development of lung fibrosis. Compared with vehicle treatment, mice exposed to nelfinavir mesylate had fewer lesions, a reduced amount of collagen deposition and reduced expression of connective tissue growth factor in the lungs.

These results highlight the therapeutic potential of nelfinavir mesylate for lung fibrosis due to its off-target inhibitory effects on fibrogenic pathways. The investigators propose taking this drug forwards for testing in patients with SSc in clinical trials.

Jessica McHugh

ORIGINAL ARTICLE Sanchez, C. G. *et al.* The antiretroviral nelfinavir mesylate, a potential therapy for systemic scleroderma. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40326 (2017)



RHEUMATOID ARTHRITIS

Do TNF inhibitors influence lymphoma development?

Arthur Kavanaugh

TNF inhibition is associated with an increased risk of lymphoma development. But is this association due to the TNF inhibitors themselves or the patient's underlying disease?

Refers to Mercer, L.K. *et al.* Spectrum of lymphomas across different drug treatment groups in rheumatoid arthritis: a European registries collaborative project. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211623 (2017)

The introduction of novel therapies, particularly biologic agents, has dramatically altered the treatment approach to rheumatoid arthritis (RA) as well as for other autoimmune systemic inflammatory conditions[1]. The resounding success of clinical trials of TNF inhibitors and subsequently other biologics led to their introduction and eventual widespread use in clinical practice, resulting in key alterations in treatment approaches and goals. Along with the clear improvements in treatment efficacy, recent years have also witnessed an evolution in the way rheumatologists regard safety. In a new study, Mercer *et al.* attempt to answer one aspect of the long-standing question of whether such therapies increase the risk of lymphomas, by focusing specifically on the types of lymphoma that develop among patients with RA, including those on TNF inhibitors[2].

Decades ago, when parenteral gold compounds were the cornerstone of RA therapy, drug safety was much simpler than today: rheumatologists would ask the patient about rashes, check their urine for proteinuria and monitor their complete blood count for thrombocytopenia. When methotrexate was introduced, the focus of drug safety evolved to include monitoring liver function tests. But with the introduction of biologics, safety considerations surrounding drug therapy expanded. Rheumatologists had to become familiar with the most appropriate screening strategies for tuberculosis and other opportunistic infections; they had to learn to watch for other adverse effects related to therapy, such as demyelinating neuropathies and inflammatory skin lesions; and finally, they had to consider the risk of cancer.

For decades, rheumatologists had recognized that patients with RA were at a higher risk of some types of cancer than the general population. Whereas some therapies were already potentially associated with certain cancers, such as methotrexate with Epstein–Barr virus-related lymphoproliferative disease, this issue grew exponentially with the advent of biologic therapies. The very name 'tumor necrosis factor inhibitor' invites consideration of cancer. Even the scientifically naive can logically follow that blocking something that kills cancer cells might increase the risk of malignancy. Of course, the truth is more nuanced. Although pro-inflammatory cytokines are an important component of host defence against malignancy, unrestrained inflammation can also increase the prevalence of certain types of cancer, such as cervical and gastric cancer[3].

In the clinic, patients frequently question whether a treatment is going to give them cancer and sometimes mention lymphoma specifically, owing no doubt, at least in part, to the surfeit of materials available on the Internet. Safety is always a tricky discussion. Although a drug can certainly be proven unsafe, it cannot really be proven safe. Safety is earned, patient-year after patient-year of exposure. It can be said that TNF inhibitors, with two decades of clinical experience and millions of treated patients worldwide, probably do not pose an increased risk of most solid tumours[4]. The situation for lymphomas is not as clear. The association between RA and the risk of lymphoma, particularly diffuse large B cell lymphoma (DLBCL), has long been recognized. Seminal work has shown that the risk of lymphoma correlates closely with disease activity[5], which is the perfect set-up for the type of bias referred to as 'confounding by indication'. The individuals at greatest risk of lymphoma are also those who are most likely to receive TNF inhibitors. Nevertheless, the development of lymphomas in patients across clinical trials of TNF inhibitors in RA has raised substantial concerns that have persisted. This suspicion is made evident by current RA guidelines that recommend choosing alternative therapies over TNF inhibitors in patients with histories of lymphoproliferative disease[6].

Optimal assessment of uncommon adverse effects comes not from clinical trials but from registries, which include larger numbers of diverse patients who are followed over longer

periods of time than in clinical trials. Data from registries continue to emerge and suggest that TNF inhibitor therapy does not increase the risk of lymphoma, providing some reassurance[6]. The study by Mercer *et al.* adds nicely to this body of literature[2]. Investigators from a dozen large RA registries from nine European countries pooled data to investigate lymphomas in patients with RA who were either exposed to various biologics or naive to such treatments. The authors confirmed earlier observations: treatment with TNF inhibitors does not seem to increase the risk of lymphoma. The investigators also went further in their investigation. Whereas immunomodulatory biologic therapies might not increase the total number of lymphomas, they might alter the types of lymphomas that develop. However, Mercer and colleagues showed that DLBCL was the most frequent type of lymphoma across all treatment exposures, militating against any effect of therapy.

> “treatment with TNF inhibitors does not seem to increase the risk of lymphoma”

The study did have several limitations. Despite having data on nearly 125,000 patients, there were not enough exposures or lymphomas across biologics other than TNF inhibitors to make definitive statements regarding these other agents. As there was heterogeneity in the processes of collecting data across registries and hence the granular data collected in each registry, some assessments relied on less than the total number of patients in each database. Nevertheless, this important study is a useful addition to the topic and can inform doctor–patient discussions.

So what is left to be done? Is the issue resolved? Perhaps not quite yet. As noted, drug safety is nearly impossible to prove, so the more strong data that are available, the better. The key missing data clinicians would like to see connects back to the original issue regarding potential bias. The association between lymphomas and RA therapy probably relates to the chronic, abundant systemic inflammation occurring in the patients who require therapy rather than the therapies themselves. Yet, in the decades since the introduction of TNF inhibitors — and some would say largely because of their use — RA is being more effectively treated than it was years ago. The rates of orthopedic surgery for RA damage seem to be decreasing, and extra-articular manifestations of disease, such as rheumatoid nodules, seem to be less frequent in the clinic[1]. Those sequelae of RA, once commonly seen in practice, also relate to chronic, abundant, systemic inflammation. One might ask why the risk of developing lymphoma has not also been decreasing if we are controlling disease activity so much more effectively. Several possible explanations could provide some explanation, the most common perhaps being that more time is required to show such a change. Clinicians who need to go to the clinic tomorrow and discuss treatment options with patients eagerly await that final piece of the puzzle.

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Competing interests statement
The author declares no competing interests.

CRYSTAL ARTHRITIS

Crystallizing our ideas about gout and osteoarthritis

Geraldine M. McCarthy and Laura Durcan

Gout and osteoarthritis are known to coexist in certain joints, with urate and calcium crystals being associated with the development of osteoarthritis. Now, research is shedding light on the depth of this association and bringing surprising observations to the fore.

Refers to Teng, G.G. *et al.* Gout and risk of knee replacement for severe knee osteoarthritis in the Singapore Chinese Health Study. *Osteoarthritis Cartilage* http://dx.doi.org/10.1016/j.joca.2017.07.017 (2017)

An association between gout and osteoarthritis (OA) has been recognized for many years, as has the clinical observation that acute attacks of gout and tophaceous deposits occur in joints affected by OA[1]. Now, a new study by Teng *et al.*[2] has used data from the Singapore Chinese Health Study, a prospective cohort of 63,257 Chinese men and women aged 45–74 years at recruitment, to assess the effect of physician-diagnosed, self-reported gout on the risk of total knee replacement. The authors of this study[2] aimed to determine a potential role for gout in the development of knee OA severe enough to require total knee replacement.

Participants in the Teng *et al.*[2] study were deemed to have gout at a follow-up telephone interview (after an average of 9.7 years) if they had been diagnosed with gout by a doctor, and if this diagnosis was based on joint pain and swelling attributed to reported hyperuricaemia. In this cohort of patients, 1,435 incidents of total knee replacement for severe knee OA were identified using the nationwide MediClaim System hospital discharge database. By this method, physician-diagnosed, self-reported gout was associated with a 39% higher risk of total knee replacement in women, but not in men, compared with individuals without gout. Surprisingly, this association was stronger in lean women (BMI <23 kg/m^2) compared with women with higher BMIs. In this study population, only 66% of patients with gout were male[2], a lower incidence than expected since, in general, the incidence of gout is twofold to sixfold higher in men than in women[3]. The 4:1 female-to-male ratio among patients undergoing total knee replacement[2] was also higher than the ratio previously observed in predominantly white populations[4]. Additionally, Chinese women tend to have a higher prevalence of knee OA than their white counterparts[2] so, although these data might not be applicable to other populations, this study[2] does draw attention to the possible links between OA and crystal arthritis.

> In contrast to OA, the progression of gout can be successfully modified…

Consistent with the data from Teng *et al.*[2] that suggested a higher incidence of OA among patients with gout, a study of 119 male patients from a US armed forces veterans' hospital found that knee OA was more prevalent in individuals with gout (68%) than in individuals with asymptomatic hyperuricaemia (52%) or age-matched normouricaemic individuals (28%)[5]. Furthermore, individuals with gout had more severe knee OA as defined by higher Kellgren–Lawrence grades, poorer self-reported functional capabilities and increased bilateral involvement compared with hyperuricaemic or normouricaemic individuals. Similarly to the Teng *at al.* study[2], the prevalence of knee OA in individuals with gout or asymptomatic hyperuricaemia was higher in non-obese men than in their obese counterparts[5].

The observations made by Teng *et al.*[2] will hopefully draw further attention to the importance of adequate management of gout. In contrast to OA, the progression of gout can be successfully modified and the associated symptoms effectively treated. Despite this fact, gaps have been consistently identified in the degree of knowledge about gout among health care providers worldwide. This knowledge gap inevitably contributes to the lack of patient education about the management of gout and is likely to contribute to poor outcomes. Therefore, despite the availability of effective urate-lowering therapies, globally, rates of urate-lowering therapy initiation and continuation and the achievement of serum urate targets are very low[6]. As current data suggest that gout seems to aggravate OA, it is extremely important that gout should be treated appropriately using urate-lowering therapies to achieve target urate levels. This strategy would reduce the burden of monosodium urate (MSU) crystal deposition and therefore diminish the effect of gouty inflammation on OA when both conditions coexist.

In light of these treatment considerations, the mechanism that underlies the association of gout with OA deserves attention. Gout can promote cartilage wear as a result of the effects of MSU crystals or soluble urate[7]. The cytokine profile seen in patients with gout seems to also be involved in OA. Activation of the NLRP3 inflammasome by MSU crystals, resulting in the release of IL-18 and IL-1β, is a key component of intense gouty inflammation[7]. Furthermore, a strong positive association exists between levels of uric acid, IL-18 and IL-1β in synovial fluid and the severity of knee OA in patients without clinical gout[7]. Stimulation of chondrocytes with IL-1β is also crucial in cartilage destruction, in which IL-1β acts by increasing production of matrix metalloproteinases (MMPs) and by impeding chondrocyte survival, and both IL-18 and IL-1β have been implicated in the pathogenesis of OA[8]. Conceivably, MSU crystals

and, perhaps, soluble uric acid could cause low-grade subclinical inflammation, which worsens cartilage degradation in primary OA and thereby contributes to the progression of knee OA.

Importantly, Teng and colleagues acknowledge the emerging body of evidence in support of a substantial pathogenic role for calcium crystals, such as calcium pyrophosphate dihydrate (CPP) and basic calcium phosphate (BCP) crystals, in OA[2]. CPP crystals can be identified in synovial fluid by polarized light microscopy, but no simple bedside test exists for intra-articular BCP crystals, as these crystals are not birefringent and are ultramicroscopic. Unlike MSU crystals, the dissolution of CPP or BCP crystals by drug therapy is not possible, nor has any drug been developed that specifically inhibits the biological activities of these crystals. Calcification of articular cartilage in the hip and knee joints is highly prevalent independent of age, but is associated with histological OA[9]. Furthermore, BCP crystals were present in 100% of cartilage samples from the knee and hip joints of patients with OA who underwent total joint replacement[9].

BCP crystals exhibit numerous biological activities *in vitro*, emphasizing their pathogenic potential in OA[10]. These activities include interaction with human chondrocytes, synoviocytes and fibroblasts to induce mitogenesis; the production of MMPs; the synthesis of prostaglandin E_2 via the cyclooxygenase pathway; the expression of proto-oncogenes; the production of nitric oxide; and the production of the pro-inflammatory cytokines IL-1, IL-6 and TNF[10]. Additionally, BCP crystals can induce osteoclastogenesis and bone resorption and inhibit anti-osteoclastogenic cytokine signalling pathways *in vitro*. BCP crystals therefore represent a potential therapeutic target in OA. Consistent with this concept, mice injected intra-articularly with BCP crystals subsequently developed synovial inflammation, cartilage degradation and increased chondrocyte apoptosis[10].

> Gout can promote cartilage wear as a result of the effects of MSU crystals or soluble urate

Overall, it is likely that the publication by Teng *et al.*[2] will help to accentuate the association between crystal-deposition diseases and OA, the most common form of arthritis in humans. Hopefully, this research will further encourage the appropriate and well-informed management of gout, and will highlight the need for further research into the pathogenic role of calcium crystals in OA and how to target them.

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VASCULITIS SYNDROMES

Tocilizumab — a new frontier for GCA therapy

Matthew J. Koster and Kenneth J. Warrington

An unmet need exists for effective glucocorticoid-sparing agents for the treatment of giant cell arteritis (GCA). Tocilizumab, the first intervention to demonstrate substantial therapeutic benefits for patients with newly diagnosed or relapsing GCA, is changing the landscape of treatment and ushering in a new era of biologic therapy.

Refers to Stone J.H. *et al.* Trial of tocilizumab in giant-cell arteritis. *N. Engl. J. Med.* **377**, 317–328 (2017)

For the past six decades, glucocorticoids have been the cornerstone of both remission-inducing therapy and maintenance therapy for patients with giant cell arteritis (GCA). Glucocorticoids suppress systemic inflammation, ameliorate symptoms and prevent ischemic optic neuropathy; however, adverse events are common, disease relapses are frequent and the treatment course can span several years. These and other factors highlight the need for therapeutic agents with a greater efficacy and lower toxicity profile than glucocorticoids. Methotrexate is the best-studied and most frequently used alternative immunosuppressant for patients with GCA, despite evidence for its efficacy being based on a meta-analysis[1] of small discordant clinical trials that demonstrated only a modest reduction in relapse risk and no lessening of glucocorticoid-induced toxicity. However, the long and arduous search for an effective glucocorticoid-sparing agent for treating GCA now seems to have finally come to fruition with the publication of the results of the GiACTA trial by Stone *et al.*[2].

Recognizing that IL-6 has an integral role in the pathogenesis of GCA, investigators pursued targeted IL-6 inhibition with tocilizumab, an IL-6 receptor α inhibitor, as a therapy for GCA; indeed, small observational studies and a recent phase II placebo-controlled clinical trial yielded preliminary signals of efficacy[3,4]. The GiACTA trial, the largest clinical trial performed to date in GCA, now provides conclusive evidence of the therapeutic benefit of tocilizumab in GCA[2]. This phase III, double-blind, placebo-controlled trial of 251 patients with GCA was the first to employ a blinded prednisone taper and a dual patient assessor strategy to avoid bias related to levels of inflammatory markers. Notably, patients were included on the basis of evidence of large-vessel vasculitis on cross-sectional imaging. Although 62% of patients in the trial had a positive temporal artery biopsy result, 37% of patients were enrolled on the basis of radiographic imaging findings, irrespective of temporal artery biopsy results[5].

> The introduction of tocilizumab as a glucocorticoid-sparing agent is a major therapeutic advance…

In the GiACTA trial[2], patients with active GCA were randomized to one of four arms: tocilizumab every week (162 mg, administered subcutaneously) plus a 26-week prednisone taper, tocilizumab every other week (162 mg, administered subcutaneously) plus a 26-week prednisone taper, weekly subcutaneous placebo plus a 26-week prednisone taper and weekly subcutaneous placebo plus a 52-week prednisone taper. The primary outcome of sustained remission at 52 weeks was significantly higher in those receiving tocilizumab weekly (56%; $n=100$) and every other week (53%; $n=50$), than in those receiving placebo with a 26-week prednisone taper (14%; $n=50$) or a 52-week prednisone taper (18%; $n=51$) ($P<0.001$ when comparing either active treatment group with placebo). Flares among all patients occurred less frequently in the groups receiving tocilizumab weekly (23%) and every other week (26%) than in the groups receiving placebo with a 26-week or 52-week prednisone taper (68% and 49%, respectively). Over the course of the 52 week trial period, patients treated with tocilizumab received approximately half of the cumulative dose of prednisone compared with the placebo groups. Overall adverse events were similar among all groups, but serious adverse events were lower among patients receiving tocilizumab, most likely owing to the reduced use of glucocorticoids in these groups compared with those receiving placebo.

Despite knowledge gained from the GiACTA trial, there remain a number of unanswered questions regarding the use of tocilizumab for GCA. For instance, the optimal duration of tocilizumab therapy is unknown, and patients often flare following tocilizumab discontinuation. Since tocilizumab directly suppresses levels of C-reactive protein, the presence of normal inflammatory markers might confuse clinical decision-making in patients with GCA who have ongoing disease activity. This situation is exemplified by a report in which a patient with GCA, who had achieved apparent clinical and biochemical disease remission when taking tocilizumab, was found to have evidence of active vascular inflammation at autopsy[6]. Although patients with ischemia-related vision loss were included in the GiACTA trial (10% of participants), those patients receiving pulse-dose glucocorticoid therapy (typically used for severe ischemic events) were not eligible for inclusion in this trial. Therefore, the results of the trial should be interpreted cautiously when considering treatment options for patients with severe ischemic events caused by GCA. Reassuringly, however, only one patient developed ischemic optic neuropathy during the GiACTA trial[2], and their vision recovered following treatment with glucocorticoids.

Another important consideration is whether treatment with tocilizumab will affect the incidence of life-threatening vascular complications caused by GCA. Despite long courses of glucocorticoids, up to one-third of patients with GCA develop thoracic aortic aneurysms, which are prone to rupture or dissection[7]. Patients with GCA undergoing aortic repair surgery are often noted to have histopathologic evidence of active aortitis many years after their initial diagnosis with GCA. Moreover, after 12 months of glucocorticoid therapy, 44% of temporal artery biopsy samples from patients with treated GCA had evidence of ongoing vascular inflammation, emphasizing the refractory nature of this type of vasculitis[8].

Over the past decade, immunologic studies have shed light on the mechanisms underlying this type of chronic vascular inflammation. Pathogenic T helper 17 (T_H17) cells, driven by IL-6 and other cytokines, are present in arteritic tissue in patients with early untreated GCA. Glucocorticoids rapidly control levels of IL-1, IL-6 and IL-23 in both the blood and in arteries affected by GCA, effectively suppressing the T_H17 cell response[9]. T helper 1 (T_H1) cells stimulated by IL-12, IL-18 and IFNγ are also important in GCA pathogenesis and are unaffected by treatment with glucocorticoids. Consequently, despite the use of high-dose glucocorticoids, chronic vasculitis is characterized by a predominant T_H1 cell signature that does not rely on the production of IL-6 (REF. 9). Similarly, although tocilizumab effectively blocks the T_H17 cell pathway, it might only have a limited effect on the chronic, IL-6-independent, T_H1-cell-mediated vasculitis. Indeed, in the GiACTA trial, flares of GCA still occurred in a quarter of patients despite treatment with tocilizumab, and almost half of patients taking tocilizumab did not reach the endpoint of sustained glucocorticoid-free remission at 52 weeks.

The reality of active disease despite IL-6 blockade highlights the fact that other cytokines and cellular mediators are integral in maintaining vascular inflammation in GCA. Alternative novel therapeutic options are beginning to emerge. In the results of a randomized clinical trial published in 2017, inhibition of T cell co-stimulatory signals with abatacept seemed to reduce the risk of relapse in patients with GCA[10]. Ongoing clinical trials are evaluating the blockade of cytokines common to both T_H17 and T_H1 cells (ustekinumab, NCT02955147) and inhibition of effector molecules downstream in their signalling pathways (baricitinib, NCT03026504).

> “…there remain a number of unanswered questions regarding the use of tocilizumab for [giant cell arteritis]”

GiACTA is undoubtedly a landmark trial that has led to the first ever FDA-approved therapy for GCA. At present however, it would be premature to consider tocilizumab as a standard-of-care therapy for all patients with GCA. It is the authors' opinion that tocilizumab, in combination with an accelerated prednisone taper, could be considered for use primarily in patients with newly diagnosed disease who are at a particularly high risk for serious glucocorticoid-associated adverse events. Tocilizumab would also be beneficial for patients with refractory or relapsing GCA who require the ongoing use of moderate to high doses of glucocorticoids. The use of tocilizumab does not seem to be warranted for patients who require very low doses of glucocorticoids to suppress minor relapses.

The introduction of tocilizumab as a glucocorticoid-sparing agent is a major therapeutic advance in treating GCA. Data on the long-term efficacy and safety of this biologic therapy are awaited, and experience with its use outside the context of a rigorous clinical trial will be informative. Meanwhile, the research agenda in GCA remains expansive. Major priorities include the discovery of specific diagnostic and prognostic biomarkers, as well as therapeutic agents that can effectively abrogate vascular inflammation and prevent long-term complications such as aortic aneurysm.

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The authors declare no competing interests.

THERAPY

Biosimilars in rheumatology — why, how and when in 2017

Roy Fleischmann

Biosimilar therapeutics for immune-mediated disease are approved in many countries and are increasingly being utilized in clinical practice. Although much has been written about the effectiveness and safety of biosimilars, less focus has been placed on why, how and when (or when not) to use these medications — until now.

Refers to Kay, J. *et al.* Consensus-based recommendations for the use of biosimilars to treat rheumatological diseases. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211937 (2017)

The advent of biologic therapies for the treatment of immune-mediated rheumatic, skin and gastrointestinal diseases has been a major advance in managing these previously difficult-to-treat diseases and has led to a striking change in therapeutic strategies[1,2]. As patents for bio-originator DMARDs have expired, multiple biosimilars of these molecules have been developed, which show a similar efficacy and safety to their bio-originators when used as the initial biologic in biologic-naive patients[3]. In a newly published consensus statement[4], Kay *et al.* attempt to answer many of the lingering questions concerning the appropriate use of biosimilar DMARDs.

The consensus statement includes five overarching principles and eight recommendations explaining why, when and how to use biosimilars[4]. The recommendations are generally supported by low-level evidence (TABLE 1); those relating to cost, extrapolation, the initial switch from a bio-originator to a biosimilar, switching from one biosimilar to a second biosimilar, and multiple switching between a bio-originator and multiple biosimilars, require further evidence and discussion before they can be fully accepted.

Current therapy with bio-originator DMARDs has two major problems: they are not effective in every patient and they are expensive. Only by producing new medications with a different mechanism of action can we solve the first problem. The cost of these drugs, however, can be enormously alleviated by the availability of biosimilars, but only if, as hoped, they are considerably less expensive than their bio-originator counterparts.

In theory, a biosimilar should cost considerably less than its bio-originator as the development costs are far cheaper[3]; if a biosimilar does not come with a major cost advantage, then there is no reason to use it. The new consensus statement[4] incompletely addresses the questions surrounding such costs; although Kay *et al.* consider cost savings to individual health systems, they do not provide a full discussion of costs to certain health care systems and to the patient. For example, the statement addresses cost savings within some single-payer systems (such as that used in Norway), in which the health care system can negotiate substantial discounts; this cost saving is theoretically passed directly on to patients and lowers the costs to the system, benefiting both the system and the patients. However, in systems in which cost savings are not passed on to the patient, and particularly in those in which the patient pays directly for the medication, unless the patient directly benefits from substantially lower costs, there is no practical reason to use the biosimilar over a bio-originator. For instance, in Japan (where patients pay 30% of their medication costs), if the medication is 50% less costly to the system, then it should be 50% less costly to the patient. However, there are many insurance systems (such as that used in the United States), in which the insurance company might benefit substantially from a reduction in cost but not pass these cost savings on to the patient, making the bio-originator and the biosimilar the same price for the patient. In this setting, why would the patient use a biosimilar?

With respect to switching and extrapolation, Kay *et al.*[4] cite the NOR-SWITCH study[5] to support their recommendations. NOR-SWITCH was an open-label study that compared switching from the bio-originator infliximab to a biosimilar infliximab in one group of patients with maintaining the bio-originator infliximab in another group. The primary outcome of this study was the non-inferiority of these two medications in patients with one of six immune-mediated diseases. Overall, the investigators concluded that the bio-originator and biosimilar were non-inferior to each other[5]. Nevertheless, the NOR-SWITCH study had many flaws, including being open-label and underpowered, with low numbers of patients in most indications and weak definitions of flare. The non-inferiority result was driven primarily by data from patients with ulcerative colitis and spondyloarthritis; the results for the other four diseases were inconclusive.

> “A key question … is whether repetitive switching … is safe and effective”

This finding raises the possibility that in the ‘real world’, even a single switch is not efficacious in a reasonable number of patients and that extrapolation to all indications might not be reasonable. The recommendations proposed by Kay and colleagues[4] could indeed be correct, but cannot be confirmed by the evidence they cite and will require future verification to be fully accepted as valid.

A key question with respect to the utility of biosimilars is whether repetitive switching between a bio-originator and a biosimilar, as well as between multiple biosimilars, is safe and effective. If so, and if multiple biosimilars of the bio-originator are available, then intense price competition and cost reduction is expected to ensue. The consensus statement[4] recommends that such switching is safe and effective; the authors based their assessment

Table 1 | **Consensus-based recommendations for the use of biosimilars**

Recommendation		Type of evidence	Grade of evidence	Requires further evidence
No.	**Description**			
1	Biosimilars must be much cheaper than their bio-originators to provide any benefit to the health care system and to the patient	Expert opinion without explicit clinical appraisal	5	Yes*
2	Biosimilars can be used to treat appropriate patients in the same way as the bio-originator	Individual RCT	1	No
3	Anti-drug antibodies to biosimilars do not need to be measured in clinical practice	Individual cohort study (including low-quality RCT)	2 or 3	No
4	Preclinical and phase I trial results should be made transparent when phase III trial results are published	Expert opinion without explicit clinical appraisal	5	No
5	Extrapolation of data on the efficacy and safety of a biosimilar from one indication to another is reasonable	Expert opinion without explicit clinical appraisal	5	Yes
6	A single switch from a bio-originator to a biosimilar is reasonable	Individual RCT	1	Yes‡
6	Switching between biosimilars is reasonable	Individual RCT	1	Yes
7	Multiple switches of biosimilars should be addressed in registries	Expert opinion without explicit clinical appraisal	5	Yes
8	No switch should be made without the knowledge of the health care provider and the patient	Expert opinion without explicit clinical appraisal	5	No

*Discussion in the consensus statement does not address this recommendation adequately for all patients. ‡Studies in the literature have conflicting results. Information for this Table obtained from Kay, J. *et al.* Consensus-based recommendations for the use of biosimilars to treat rheumatological diseases. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211937 (2017). RCT, randomized controlled trial.

on 'expert opinion' as no studies currently support this recommendation. In doing so, Kay *et al.* incorrectly assume that because no trials or reports demonstrate that switching from one biosimilar to another is unsafe or ineffective, the reverse must be true. No trials have yet addressed this strategy, so until researchers have confirmed this assumption, physicians and patients should remain cautious about multiple switches between biosimilars.

The FDA has proposed a new study design for use in clinical trials that assess whether multiple switching is safe and effective[6]. Clinical trials are more time-consuming and expensive than looking at registry data, an approach suggested by Kay *et al.*[4]; however, it is more ethical and rational to test switching (a potentially ineffective strategy with unknown safety consequences) in a well-controlled clinical trial involving a minimum number of patients, as suggested by the FDA, than in registries in which thousands of patients could be at risk and are not followed up as carefully. Registries have advantages, but researchers must also consider their numerous disadvantages: clinicians might not perform assessments (in whole or in part) on every patient at every visit, and visits might not be at regular intervals.

The key take-home message of this consensus statement[4] is that the only reason for using a biosimilar is to lower the cost of medication to society and to the patient so that as many patients as possible have access to these medications. If this objective is not accomplished, then biosimilars serve no useful purpose. Hopefully, the goal of markedly reducing the costs of biologic therapeutics will be accomplished in the near future and the questions surrounding the safety of multiple switches will be answered, addressing the doubts raised above.

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THERAPY

Are you managing osteoarthritis appropriately?

David J. Hunter and Jocelyn L. Bowden

For patients with osteoarthritis, the current palliative approach of analgesic prescription followed by joint replacement is often inappropriate. Instead, care should be tailored to the needs of individuals and targeted towards the central complaints of pain and functional limitation. So why are we still getting it wrong?

Refers to Yu, D. *et al.* Population trends in the incidence and initial management of osteoarthritis: age-period-cohort analysis of the Clinical Practice Research Datalink, 1992–2013. *Rheumatology (Oxford)* http://dx.doi.org/10.1093/rheumatology/kex270 (2017)

Osteoarthritis (OA) represents a large and increasing health burden that has substantial implications for the individuals affected and for health care systems, as well as wider socioeconomic costs. By 2020, over 500,000 hip and 1.3 million knee replacement procedures are expected to be performed annually in the USA, primarily to treat pain and functional limitations resulting from OA[1]. By 2030, an estimated 25% of the population of the USA (67 million adults) will have OA, of whom 25 million will experience limitations in activity caused by this disease[2]. In addition, the societal costs of OA currently equate to 0.25–0.50% of the GDP of most developed countries[3]. There is no shortage of well-developed guidelines for the management of OA, yet the management of OA does not typically correlate with these guidelines, suggesting that the majority of patients with OA are not receiving appropriate care[4]. This discordance has been reinforced by a new study by Yu *et al.*[5], which also identified the continued use of radiography for diagnosis and opioids as therapy for OA as points of concern.

Current management practices for OA are not adequately focused on optimizing patient outcomes or reducing unsustainable increases in healthcare costs. Clinicians underutilize efficacious evidence-based lifestyle behaviour management strategies such as exercise and weight loss[6] in favour of expensive and reactive treatments. These palliative treatments frequently have no clinically meaningful benefit over placebo and are often harmful, as well as not being cost-effective[7]. Robust evidence suggests that glucosamine, paracetamol, opioids, viscosupplementation and arthroscopy, among other popular OA interventions, fall into this category, as reflected by their reduced stature in guidelines. In addition, the majority of patients with OA have two or three affected large joints, yet many health care systems currently incentivize expensive and invasive interventions that might only benefit one joint. Supporting or subsidizing services to improve behavioural changes that are centred on exercise and weight loss would provide a more holistic approach to OA management at a much smaller cost. The discordance between clinical practice and guidelines is thought to be a result of the common trivialization of OA as a condition of normal ageing, coupled with the perception that treatment options are limited. These misconceptions could be addressed by improving the dissemination and implementation of evidence-based care.

Although slightly more conservative than other reports, the study by Yu *et al.*[5] confirmed that rates of clinical OA are rising. The authors used a large administrative database (Clinical Practice Research Datalink) to examine trends in the rate of new cases of OA and in the management of patients with newly diagnosed OA in primary care in the UK. As with any administrative health care database, the authors recognized important limitations, including the precision with which OA is diagnosed, the completeness of medical records and the potential for generalizing the data to the wider population.

The results of this study[5] provide important insights, prompting reflection on current practice and suggestions for further improvements in health care service delivery and health resource utilization. First, the increasing incidence of clinical OA mirrors that of obesity and foreshadows a future increase in demand for joint replacement surgery. The presence of multiple long-term conditions and the use of multiple medications are common in patients with OA, with the majority of such individuals reporting using five or more unique categories of drug in the past year[5]. Intriguingly, the incidence of hand OA observed by Yu *et al.*[5] was substantially lower than is typically found in epidemiologic investigations. Despite studies highlighting the evident disability associated with hand OA, a substantial proportion of people with hand OA still might not be presenting to clinical care. Another important finding is that the average age at diagnosis falls within 1–2 years of the mean age at which individuals undergo arthroplasty for OA, suggesting that patients are presenting to physicians for the first time at a late stage in their disease course.

Other departures from current guidelines noted by Yu *et al.*[5] were the high degree of usage of plain radiographic imaging for diagnosis and a shift towards the prescription of opioids as analgesics. Imaging is recommended only to help confirm a diagnosis in people with atypical presentations[8], yet patients can focus unnecessarily on imaging features that typically bear a poor correlation to their symptoms. Similarly, opioids are of little benefit to patients with OA, carry a substantial risk of harm and are not cost-effective[9]. If considered at all, such as for patients averse to total knee arthroplasty, opioids should be prescribed on a short-term basis, with clear goals and a regular review of treatment response and adverse effects.

In light of the results of the Yu *et al.*[5] study, what should be done? Great opportunities exist for enhancing the delivery of efficacious treatments, and developments in health care delivery could favourably influence future care. International trends of

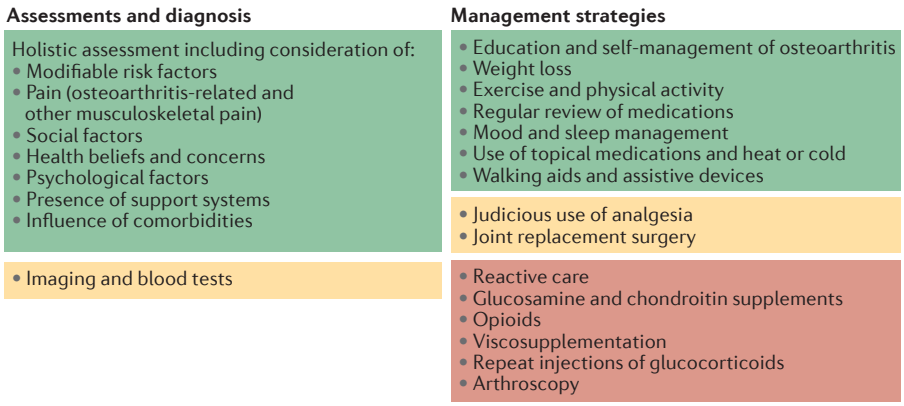

Figure 1 | **Principles for the diagnosis and management of osteoarthritis.** Standards to aim for (green boxes) in the diagnosis and management of osteoarthritis and practices that should be limited (orange boxes) or discouraged (red boxes) are highlighted. General principles for management should include coordinated care, the setting of individual goals, detailed follow-up and the use of a multidisciplinary model of care.

increasing levels of obesity and ageing populations, along with rising injury rates, will lead to further increases in the incidence of OA. Ideally, a proactive society would focus on the two foremost risk factors for OA development — obesity and joint injury — both of which are modifiable. Failing that, society will be left with rising levels of chronic disease with a concomitant substantial personal burden, alarming amounts of direct health care expenditure and indirect economic consequences.

The formulation of a diagnosis and management plan for a patient with OA should start with a holistic assessment, rather than a reliance on imaging. The performance of unnecessary imaging and the dependence on such to confirm what is already indicated clinically is both financially costly and redundant. Such investigations should contribute to management; yet, in a substantial proportion of individuals with knee pain, imaging does not favourably or meaningfully influence management choices.

Care for patients with OA should be tailored to individual needs and goals. Decision-making should be based on the best possible evidence, with patient safety, access to information and a proactive anticipation of patient needs prioritized over a reactive health service. Patient responses to therapy should be monitored on a regular basis to ascertain adherence and uptake of recommendations, as well as to encourage ongoing behaviour modification and to identify potential issues. Important factors that should be considered by the clinician include a patient's knowledge of OA and previous treatments, their current level of pain and functional impairment, mood and sleep disturbance, the presence of comorbidities and the patient's expectations of treatment. The nihilistic approach of many health professionals to OA and our inability to diagnose OA at an early stage reduces the opportunity to provide meaningful therapeutic benefit early in the disease process.

Management should prioritize interventions with the strongest evidence and provide the greatest benefit with minimal risk and cost (FIG. 1). Health care systems should encourage the delivery of interventions that improve a patient's knowledge of OA and their ability to self-manage the disease, tailoring management to the individual needs of the patient, which should be targeted towards the central complaints of pain and functional limitation. Management practices should shift toward optimizing treatment efficacy using a cohesive multidisciplinary approach. This model of health care delivery is supported by many policy changes made over the past few years and will facilitate improved patient outcomes while reducing inappropriate health care utilization and wasting of resources[10].

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OSTEOARTHRITIS

In search of phenotypes

Sita M. Bierma-Zeinstra and Marienke van Middelkoop

Identifying different phenotypes of osteoarthritis is currently a subject of much research; however, a new systematic review has sparked discussion about the discrepancies in how research into disease phenotypes is conducted. Can we define individual phenotypes if we cannot agree on what constitutes a phenotype in the first place?

Refers to Deveza, L. A. *et al.* Knee osteoarthritis phenotypes and their relevance for outcomes: a systematic review. *Osteoarthritis Cartilage* http://dx.doi.org/10.1016/j.joca.2017.08.009 (2017)

Knee osteoarthritis (OA) is regarded as a heterogeneous disease with multiple aetiologies. Considering the modest effectiveness of a wide range of treatments for OA and the variable progression of OA, the call for well-defined phenotypes of knee OA is obvious. The identification of such phenotypes or subgroups could help us to better understand the driving factors in the development and progression of OA and to define subgroup-specific treatments to improve therapeutic effectiveness[1]. Although patients with some subgroups of knee OA already receive targeted treatments in clinical practice (such as varus-aligned medial knee OA), there is no generally accepted classification system for OA phenotypes, as illustrated by the findings of Deveza *et al.*[2] in a new systematic review.

Deveza *et al.*[2] analysed data from 34 observational studies of patients with symptomatic knee OA that aimed to identify subgroups of the disease based on any OA characteristic and assessed these studies for their association with clinically important outcomes. Pain sensitization, psychological distress, radiographic severity of OA, BMI, muscle strength, inflammation and comorbidities were associated with poor clinical outcomes, and male sex, the pattern of cartilage damage, inflammation, obesity and other metabolic abnormalities were associated with poor structural outcomes[2]. The authors emphasized that the majority of the studies included were cross-sectional, defined phenotypes on the basis of a single phenotypic characteristic and did not investigate external validity; however, some studies were retrospective and identified longitudinal trajectories on the basis of progression. Deveza *et al.* also regarded these trajectories as phenotypes and associated them with baseline characteristics.

Similarly, in a 2016 systematic review, Dell'Isola *et al.*[3] analysed data from 25 studies (12 of which were also included in the Deveza *et al.*[2] study) that aimed to identify knee OA phenotypes and used analyses or methodology specifically designed for this purpose (such as cluster analysis). Dell'Isola *et al.* extracted key variables for each phenotype presented in these underlying studies and assigned these variables to predefined categories. The authors proposed several phenotypes: chronic pain-associated OA (in which central mechanisms are prominent); inflammation-associated OA; metabolic syndrome-associated OA; OA associated with joint-localized bone and cartilage metabolism; mechanical load-associated OA; and OA with minimal disease and a low rate of progression[3]. The wide range of studies included in both systematic reviews[2,3], with different outcomes, characteristics and methodologies, as well as the different approaches used by their authors, illustrates the non-standardized nature of research into detecting and defining phenotypes.

When searching for knee OA phenotypes, there are some essential issues that must be considered. First, there needs to be an agreement on when a subgroup of patients can be said to have a specific phenotype. For example, can a phenotype be based on a single factor if this factor is predictive of outcome? And, is a phenotype based on several factors only meaningful when those factors interact and are able to predict prognosis or treatment response better when used together than separately? In our opinion, disease phenotypes are meaningful when they reflect differential treatment effects, prognosis or aetiology, regardless of whether they are based on a single factor or on multiple factors.

Only six of the studies included by Deveza *et al.*[2] assessed the association between OA phenotypes and disease prognosis, and many of these six only investigated a single factor. Although this systematic review found only limited evidence for the prognostic value of such phenotypic characteristics, other systematic reviews of knee OA (using data from >30 studies) have highlighted the prognostic value of single factors for clinical progression[4] and for structural progression[5]. These reviews revealed strong evidence for an association between multiple factors and either clinical or structural progression, suggesting that such prognostic factors could also be considered to be phenotypes.

Phenotypes described in the cross-sectional studies included by Deveza *et al.*[2] were often based on multiple factors, but these factors mostly occurred within one dimension of disease (for example, within a psychological profile or a comorbidity profile). Whether these phenotypes predict prognosis or response to certain treatments and whether they do so in a better way than phenotypes based on separate underlying factors remains to be seen.

In their systematic review, Deveza *et al.* considered groups of patients with different trajectories of progression to be separate phenotypes[2]; however, whether phenotypes should be based on outcome is a matter of debate. A phenotype that is based on characteristics of OA seen during clinical consultation would seem to be more meaningful than one based on outcomes, as this kind of classification might affect treatment decisions. To increase the relevance of identified phenotypes to clinical or structural outcomes, Deveza *et al.* limited their search to studies that assessed the association of a subgroup or phenotype with clinically important outcomes[2]. Studies that associated different aetiological factors or risk factors with identified phenotypes

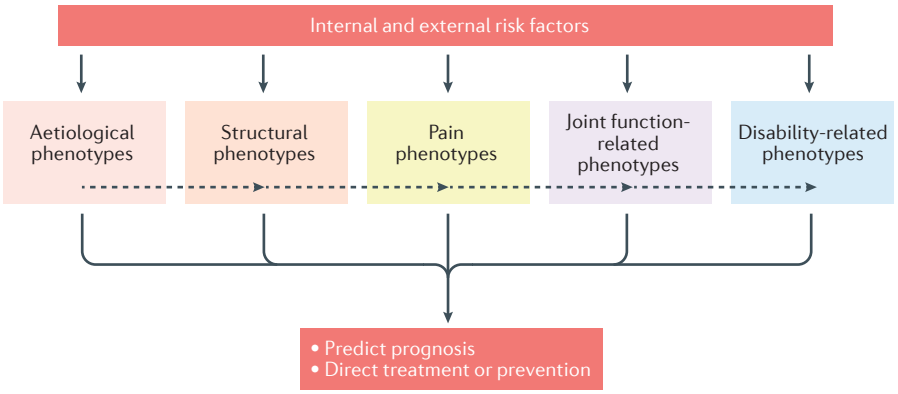

Figure 1 | **Defining phenotypes of osteoarthritis based on dimensions of disease.** The different dimensions of osteoarthritis can be used to define disease phenotypes. Dotted lines indicate the potential for interrelation between the dimensions of disease.

were therefore excluded; however, such studies could provide clues to direct phenotype-specific preventions or disease-modifying treatments.

Deveza *et al.*[2] also deliberately excluded studies that assessed the influence of subgroups or phenotypes on the effect of treatments in randomized controlled trials (RCTs), yet testing and applying phenotype-targeted treatments seems to us to be the ultimate goal of defining phenotypes of OA. Although some therapies are already specifically designed for and tested in certain subgroups of patients, for other therapies, researchers try to identify subgroups of patients who respond in different ways to treatment using subgroup analysis. The frequently reported and often underpowered *post hoc* subgroup analysis of RCTs is usually regarded as useful only for generating hypotheses; reliable subgroup analyses need to fulfil certain methodological criteria, such as being predefined and adequately powered[6–8].

An initiative utilizing the OA Trial Bank aims to report on treatment effects in subgroups of patients with OA in an appropriately powered way by combining intervention-wise individual patient data from multiple RCTs[7]. Such an approach should enable an assessment of the effect of treatments on subgroups of patients with OA, according to predefined hypotheses by meta-analysis of individual patient data[7]. The reality, however, is that not all RCTs measure potential subgroup characteristics of interest and the RCTs that do, measure them differently to each other. The challenge is to ensure that the same minimal and feasible set of phenotypic characteristics with a high potential for differentiating subgroups based on response to treatment are measured in RCTs. In the 2015 Osteoarthritis Research Society International (OARSI) Clinical Trials Recommendations, a set of baseline characteristics was recommended for use when describing a study population[9]. This set of characteristics should probably be critically reassessed on the basis of potentially important phenotypic characteristics, including those reported by Deveza *et al.*[2] in the next update of these recommendations. A similar minimal set of characteristics should be used for knee OA cohort studies.

A main conclusion that can be drawn from the current systematic reviews on OA phenotypes[2,3] is that patients with OA can be divided into subgroups or phenotypes on the basis of many different dimensions of the disease, such as phenotypes based on aetiological grounds, structural features or symptomatic presentation (for example, OA in which chronic pain mechanisms are important). In our opinion, phenotypes of OA in different dimensions of disease can exist side by side because they can serve different purposes: from choosing symptomatic treatment, to predicting prognosis, to developing and testing different disease-modifying or preventive treatments (FIG. 1). A search for phenotypes that include multiple different dimensions of disease might be a step too far, as such dimensions do not necessarily associate with each other; however, the relationship between such dimensions should be investigated. Furthermore, identifying mutually exclusive phenotypes is contrary to the nature of a multifactorial disease like OA. The challenge now is to agree on the dimensions of disease, to find the phenotypes within these dimensions that are relevant to diagnosis, prognosis and therapy, and to identify the most discriminative factors for these phenotypes that can easily be used and tested for in clinical practice.

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Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment

Joachim R. Kalden[1] and Hendrik Schulze-Koops[2]

Abstract | The availability of monoclonal antibodies has revolutionized the treatment of an increasingly broad spectrum of diseases. Inflammatory diseases are among those most widely treated with protein-based therapeutics, termed biologics. Following the first large-scale clinical trials with monoclonal antibodies performed in the 1990s by rheumatologists and clinical immunologists, the approval of these agents for use in daily clinical practice led to substantial progress in the treatment of rheumatic diseases. Despite this progress, however, only a proportion of patients achieve a long-term clinical response. Data on the use of agents blocking TNF, which were among the first biologics introduced into clinical practice, provide ample evidence of primary and secondary treatment inefficacy in patients with rheumatoid arthritis (RA). Important issues relevant to primary and secondary failure of these agents in RA include immunogenicity, methodological problems for the detection of antidrug antibodies and trough drug levels, and the implications for treatment strategies. Although there is no strong evidence to support the routine estimation of antidrug antibodies or serum trough levels during anti-TNF therapy, these assessments might be helpful in a few clinical situations; in particular, they might guide decisions on switching the therapeutic biologic in certain instances of secondary clinical failure.

The availability of antibody constructs including monoclonal antibodies and, more recently, bispecific antibodies[1] has revolutionized the treatment of diseases for which no efficacious medication existed in the past, such as chronic inflammatory and autoimmune diseases. Although treatment with such antibody constructs was first attempted in transplantation medicine[2,3], clinical trials with monoclonal antibodies were performed on a larger scale in the 1990s by rheumatologists and clinical immunologists, followed by the approval of these biologic treatments for use in daily clinical practice[4–9]. However, despite this substantial progress in the treatment of rheumatic diseases, such as rheumatoid arthritis (RA) and spondyloarthritis (SpA), only 60–70% of patients with these diseases achieve a long-term clinical response[5,7–9]. A certain proportion of patients will not respond to treatment with a biologic agent at all (primary failure) or, following an initial good response, the clinical efficacy of the biologic will be lost over time (secondary failure)[10,11]. In addition, some patients have to discontinue treatment with a given biologic because of adverse events, most notably infections[12].

Several biologics with different targets have been introduced in the treatment of inflammatory rheumatic diseases. TNF antagonists were the first clinically successful biologics, and experience with these agents is consequently more extensive than with biologics with a different mode of action. In the present Review, therefore, we discuss several critical issues related to primary and secondary failure of TNF antagonists in patients with inflammatory rheumatic diseases, with a focus on RA, including the possible role of antidrug antibodies (ADAs), methodological problems for their detection and their effect on treatment strategies. Finally, we outline several recommendations regarding when a patient should be tested for the presence of ADAs or trough serum levels of a biologic. As most of the relevant data have been collected for the first TNF antagonists approved for clinical use, namely infliximab, adalimumab and etanercept, this Review concentrates mainly on these three compounds; brief comments on other anti-TNF agents are made only where sufficient data are available.

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Key points

- Monoclonal antibodies and fusion proteins induce the formation of antidrug antibodies (ADAs), the occurrence and magnitude of which differs between chimeric antibodies, fully humanized antibodies and fusion proteins
- The clinical implication of ADAs are unclear, although ADAs are known to cause secondary drug failure
- Consensus definitions of primary and secondary non-response are lacking, as are evidence-based recommendations on how to guide biologic therapy on the basis of drug level and immunogenicity
- Testing for ADAs or serum trough drug levels might be indicated in some situations of primary and secondary treatment failure and could prompt changes in dosing or therapeutic agent
- When switching from an anti-TNF drug (originator) to a biosimilar of that originator, one has to take into consideration that ADAs against the originator will crossreact with the biosimilar, causing treatment failure
- More information regarding the immunogenicity of the different TNF antagonists and improved ADA testing systems could lead to the development of an immune-pharmacologic strategy for the individualized treatment of rheumatoid arthritis

Drug survival of TNF antagonists

Drug survival, or the time to discontinuation of a drug, is influenced by various factors such as lack of efficacy, loss of efficacy (possibly owing to immunogenicity), adverse events and poor adherence, among others. Various large national registries of patients with RA receiving biologic therapy provide valuable data on drug survival rates. Data from an Italian registry[13] showed that etanercept survival was better than that of infliximab or adalimumab over 4 years of observation. Factors that predicted drug continuation included the use of concomitant conventional synthetic DMARDs (csDMARDs), mainly methotrexate, and the presence of comorbidities, but not high disease activity scores. In a Dutch study, drug survival did not differ among these same three TNF-blocking agents[14]. In a study using data from the Danish DANBIO registry[15], infliximab had the lowest rate of treatment response, disease remission and drug survival, whereas adalimumab had the highest rates of treatment response and disease remission and etanercept showed the highest drug survival rate. Data from the Brazilian BIOBADABRASIL registry[16] showed that, over an observation period of up to 4 years, anti-TNF drug survival in patients with RA was 47.5 months (95% CI 45.65–49.36), which was significantly lower than the drug survival duration observed in patients with ankylosing spondylitis (AS) (63.1 months; 95% CI 60.4–65.9). In both diseases, the main causes of discontinuation were ineffectiveness and adverse events. Similar data were reported from the Spanish BIOBADASER registry, where again drug survival was longer in patients with AS than in those with RA[17]. Finally, in an Italian study[18], the overall 10-year retention rate of first-line anti-TNF agents was ~23%, and was higher in patients with SpA than in patients with RA (30.5% versus 20.4%). Furthermore, etanercept was found to be the most persistent anti-TNF medication, with a higher drug survival rate than that of infliximab and adalimumab.

Notably, these data sit in contrast to those from a retrospective study performed in a single rheumatology department, in which no differences were found between the survival rates of the TNF antagonists adalimumab, infliximab and etanercept in the treatment of 770 patients with inflammatory rheumatic diseases in daily clinical practice[19]. The reasons for this discrepancy are difficult to discern and could relate to a controlled versus 'real life' population in the rheumatology department and the registries, respectively, resulting in different frequencies of patients taking methotrexate despite seemingly similar rates of methotrexate prescription.

Primary failure of TNF antagonists

There is currently no consensus regarding the definition of a primary non-response to an anti-TNF drug. Primary lack of efficacy might be recognized if a patient never responds to a newly initiated TNF inhibitor or fails to respond within 16 weeks[20,21] and it has been suggested that patients could be classified as 'responders' if they achieve a reduction in the 28-joint disease activity score (DAS28) of ≥1.2 from baseline[22,23]. A longer disease duration and a higher disease activity before commencement of TNF inhibitor therapy have been proposed to increase the risk of primary non-response[23]; however, the reasons for primary failure of TNF antagonists are still incompletely understood. Furthermore, as it is well known that RA is a heterogeneous disease with variability in the inflammatory infiltrates in the synovial membrane[24,25], it seems possible that the main pathogenic mechanisms might be different in certain subsets of patients, leading to different clinical responses. In this context, the demonstration of a paradoxical expansion of T helper 1 (T_H1) and T_H17 lymphocytes in patients with RA following treatment with infliximab is of interest, as this phenomenon might also explain a lack of clinical responsiveness in a minority of patients with RA[26]. TNF interferes with cell trafficking, as exemplified in a 2009 study by Souto-Carneiro *et al.*, which indicated that the trafficking of memory B cells into inflamed tissue is regulated by TNF[27]. In addition, a high BMI might diminish the primary response to TNF antagonists[28]. Likewise, at least in patients with Crohn's disease, smoking has also been demonstrated to have a strong adverse effect on the rate and maintenance of response to infliximab[29].

A number of studies have also explored the possible association of serological or genetic markers with the response to TNF antagonists. Thus, the presence of rheumatoid factor or anti-cyclic citrullinated peptide antibodies can be associated with a reduced response to anti-TNF drugs; however, these antibodies account for only a small proportion of the variance in treatment responses[30]. Considering the various publications on the association of genetic factors with response to treatment and outcome in RA, it seems fair to say that, to date, no parameters are available to identify those patients who could be predicted to have a primary failure when treated with TNF antagonists. A French study showed that patients with RA and a TNF-308 G/G genotype have a better response to infliximab than those with A/A or A/G genotypes; the authors suggested that TNF-308 genotyping might be a useful tool for predicting response to infliximab therapy[31]. In the Norfolk Arthritis Register (NOAR),

the *HLA-DRB1* locus was associated with treatment response in RA, as well as with disease susceptibility, radiological severity and mortality[32]. Of note, a number of genotyping studies in RA indicate that the genetic and epigenetic predictors of responsiveness to treatment might differ depending on the TNF inhibitor used. However, one has to take into consideration that many of the genetic associations reported have still to be validated in different patient cohorts and also that genotyping is not available, at least at present, for routine use in clinical practice[33–39]. In addition, the introduction of genome-wide association studies as well as gene expression studies to identify genetic predictors of treatment response might be further complicated by the emergence of different genetic patterns depending on the biologic used, including TNF antagonists. Of interest, a 2015 report indicated that human immunoglobulin allotypes in the IgG1 heavy chain (G1m1 and G1m17 allotypes) are associated with response to infliximab and could improve therapeutic targeting in patients with RA[40].

With regard to the use of therapeutic monoclonal antibodies, the specific molecule of the antibody and the dosing schedule are also relevant when analysing reasons for a lack of efficacy. Monoclonal antibodies have complex pharmacokinetics and pharmacodynamics, which are dependent in part on the structure of the antibody as well as on the structure of the target antigen. Depending on the target antigen, monoclonal antibodies might exhibit linear or nonlinear pharmacokinetic behaviour, with the latter situation due to receptor-mediated clearance. In the case of nonlinear kinetics, clearance can be altered owing to receptor loss following repeated doses and is possibly associated with disease severity[41,42]. Even today, it is sometimes difficult to choose the right treatment dose for a particular patient. If treatment doses are calculated from experimental animal models, the possible existence of interspecies differences in pharmacology also has to be taken into consideration.

Secondary failure of TNF antagonists

Effectiveness of a TNF inhibitor might be lost over time despite a good initial response. As is the case with primary failure, there is currently no consensus regarding the definition of a secondary response failure, although it has been suggested that secondary treatment failure might be considered if there was an increase in DAS28 of >0.6 during the previous 6 months or an increase in the EULAR disease activity score[21,22].

Loss of clinical efficacy is the main reason for discontinuation of anti-TNF therapy. For example, in a Brazilian study investigating the reasons for discontinuation of anti-TNF medication in 304 patients, ineffectiveness was the cause in 48.35% of the patients, adverse events in 34% and other reasons in 17.10%[16]. Similar data were reported from an Italian study, where 45% RA patients discontinued anti-TNF therapy because of lack of efficacy[18]. A systemic review and meta-analysis of drug registries in health care databases found that a lack of efficacy was the primary cause of discontinuation of antibody therapy[43]; these data were confirmed by studies from Switzerland[11] and Korea[44].

As is the case with trying to identify patients who will experience primary failure of anti-TNF treatment, at present there are no parameters available that can reliably predict a discontinuation of TNF inhibitors in patients with RA[45,46]. The clinical features that are consistent with a secondary loss of response are the re-emergence of symptoms such as synovitis. Furthermore, as already mentioned, an increased BMI[28,29] and, in the case of Crohn's disease, smoking might have a strong adverse effect on the rate and maintenance of response to infliximab[29].

Antidrug antibody formation

Most biologic drug products elicit some level of immune response, which can lead to serious adverse effects or loss of efficacy. The formation of ADAs can thus affect drug survival, particularly as a cause of secondary response failure.

The various TNF antagonists (which have different molecular structures (FIG. 1)) differ in their dosing regimens, routes of injection, pharmacokinetic properties and immunogenicity[47]. The quantification and comparison of the immunogenicity of these TNF antagonists are largely dependent on the assay used to detect the ADAs. However, some general conclusions can be drawn concerning the immunogenicity of infliximab, etanercept and adalimumab, as reviewed elsewhere[48]. Infliximab is the most immunogenic TNF antagonist, as compared with adalimumab and etanercept, particularly when it is used without concomitant methotrexate[47,48]. Of interest, ADAs can bind to the idiotope (for example, the antigen-binding region) of a therapeutic monoclonal antibody, such as that of the fully human monoclonal antibody adalimumab and the chimeric monoclonal antibody infliximab. As a receptor construct, etanercept does not have an idiotype (that is, it does not express idiotopes), which might explain in part why etanercept is associated with a reduced immunogenicity[48].

Infliximab, etanercept and adalimumab all neutralize soluble TNF; recent data suggest that differences between these TNF antagonists in their neutralization capacities depend on the concentration of soluble TNF[48]. All three also bind to transmembrane TNF (tmTNF). Current evidence suggests that TNF antagonists have a dual function and can act as antagonists by blocking TNF interactions with the TNF receptors TNFR1 and TNFR2, or by initiating a reverse signalling cascade that leads to apoptosis, cell activation or cytokine suppression[48]. Which of these two mechanisms of action is the more important remains to be determined. Along this line of investigation, a study published in 2017 indicates that tmTNF is transiently expressed on the surface of lipopolysaccharide-stimulated primary human monocytes, macrophages and monocyte-derived dendritic cells, and that expression of tmTNF on the cell surface is enhanced following treatment of cells with a TNF convertase (TACE) inhibitor[48,49]. Binding of anti-TNF biologics to tmTNF on dendritic cells resulted in a rapid internalization of the anti-TNF–tmTNF complexes, leading to the production of anti-TNF peptides that could leach from the surface of dendritic cells. Whether this intriguing mechanism could lead to the development of ADAs and thus to anti-TNF neutralization is still unclear[49].

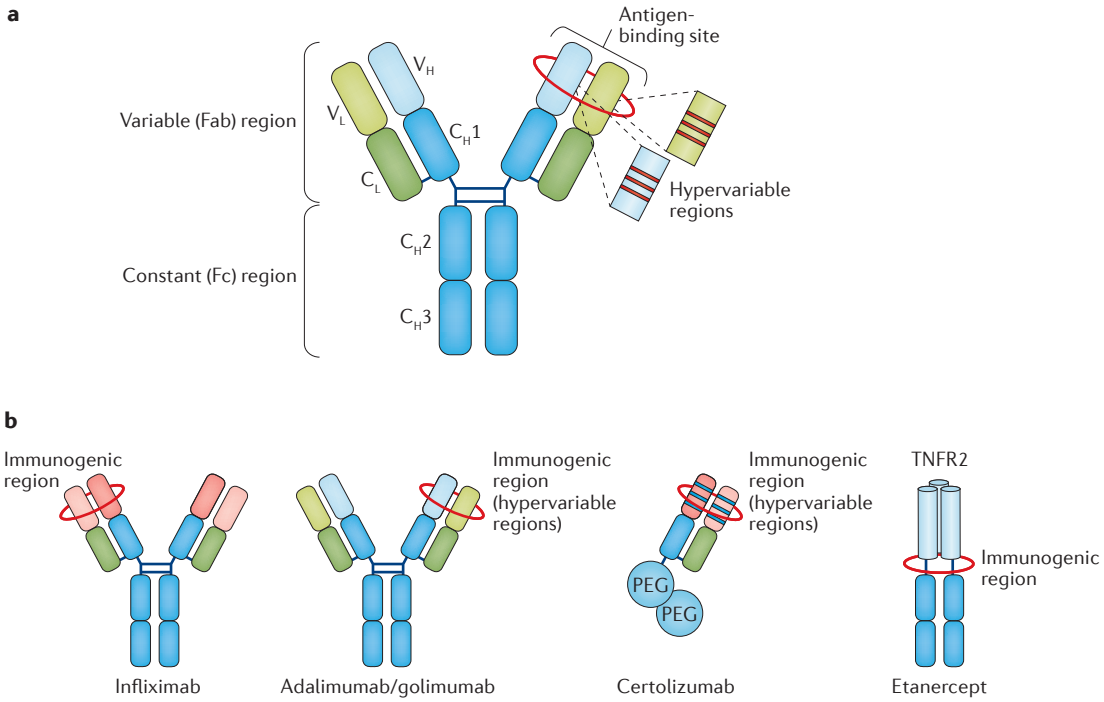

Figure 1 | **Structure of TNF antagonists. a** | Schematic representation of an antibody molecule. The Fc region is responsible for the effector functions of the antibody, and the Fab region forms the antigen-binding site. Within the variable regions are small areas of hypervariability, which determine antigen specificity. **b** | Anti-TNF antibody constructs used in the treatment of rheumatoid arthritis. Infliximab is a chimeric monoclonal antibody with a murine variable region (shown in red) fused to a human Fcγ1 Ig. Adalimumab and golimumab are fully human monoclonal antibodies. Certolizumab is a humanized Fab' fragment bound to polyethylene glycol (PEG) molecules. Etanercept is a TNF receptor–Fcγ1 fusion protein. Potentially immunogenic areas within each antibody construct are indicated in red. Abbreviations: C_H, constant heavy; C_L, constant light; TNFR2, TNF receptor 2; V_H, variable heavy; V_L, variable light.

ADAs might lead to local or systemic adverse effects. ADA-induced adverse events include acute infusion reactions and delayed infusion reactions, as well as disseminated skin reactions such as maculopapular exanthema[50,51]. The pathogenic mechanisms leading to acute reactions are not yet fully understood. Antibody-mediated reactions could involve complement-mediated events as well as the release of cytokines following the binding of the biologic to Fcγ receptors on immune cells[52]. Systemic infusion reactions might involve the formation of immune complexes, and IgE antibody formation might be responsible for adverse effects[53–55].

A possible role of ADAs has been suggested as a reason for the differences in drug survival observed in various studies, but this issue has not been unequivocally resolved. In particular, it is surprising that in some studies the drug survival of infliximab and adalimumab in SpA was higher than in RA[18,44], although in SpA, TNF inhibitors are usually given without concomitant immunosuppressive therapy (such as methotrexate). The differences in the development of ADAs in SpA as compared with RA might be related to differences between the two diseases in the overall degree of systemic inflammation. Certainly, in RA systemic inflammation is substantially increased, with an increased B cell proliferation fostering the production of ADAs; by contrast, the systemic inflammatory activity in SpA is rather limited. It is tempting to speculate that, in a condition like SpA, the immunosuppressive effect of TNF inhibitors is sufficient to prevent the induction of an immune response against the therapeutic protein, whereas in RA the inflammatory cytokine milieu instead facilitates an ADA response.

Testing for antidrug antibodies

Different methods can be used to detect and to characterize ADAs (FIG. 2). Some direct and indirect enzyme-linked immunosorbent assays (ELISAs) are relatively inexpensive; however, they might be prone to false-positive results and nonspecific binding. Improved methods include the two-side or bridging ELISA and the radioimmunoassay antigen binding test, although both systems have their own pitfalls. Bridging ELISA is susceptible to drug interference by the monoclonal antibody and typically measures ADAs only in the absence of detectable drug levels. In addition, this test system cannot quantify IgG4 antibodies to adalimumab[56]. By contrast, the radioimmunoassay can capture clinically relevant IgG1 and IgG4 ADAs[56]. The detection of ADAs is additionally confounded by the presence in the serum of high concentrations of rheumatoid factor and by the presence of the drug itself[56]. Interference with the available test systems might also arise from the processing and presentation of biologic-derived antigens to T cells and by complex formation, including the involvement of the complement system[57].

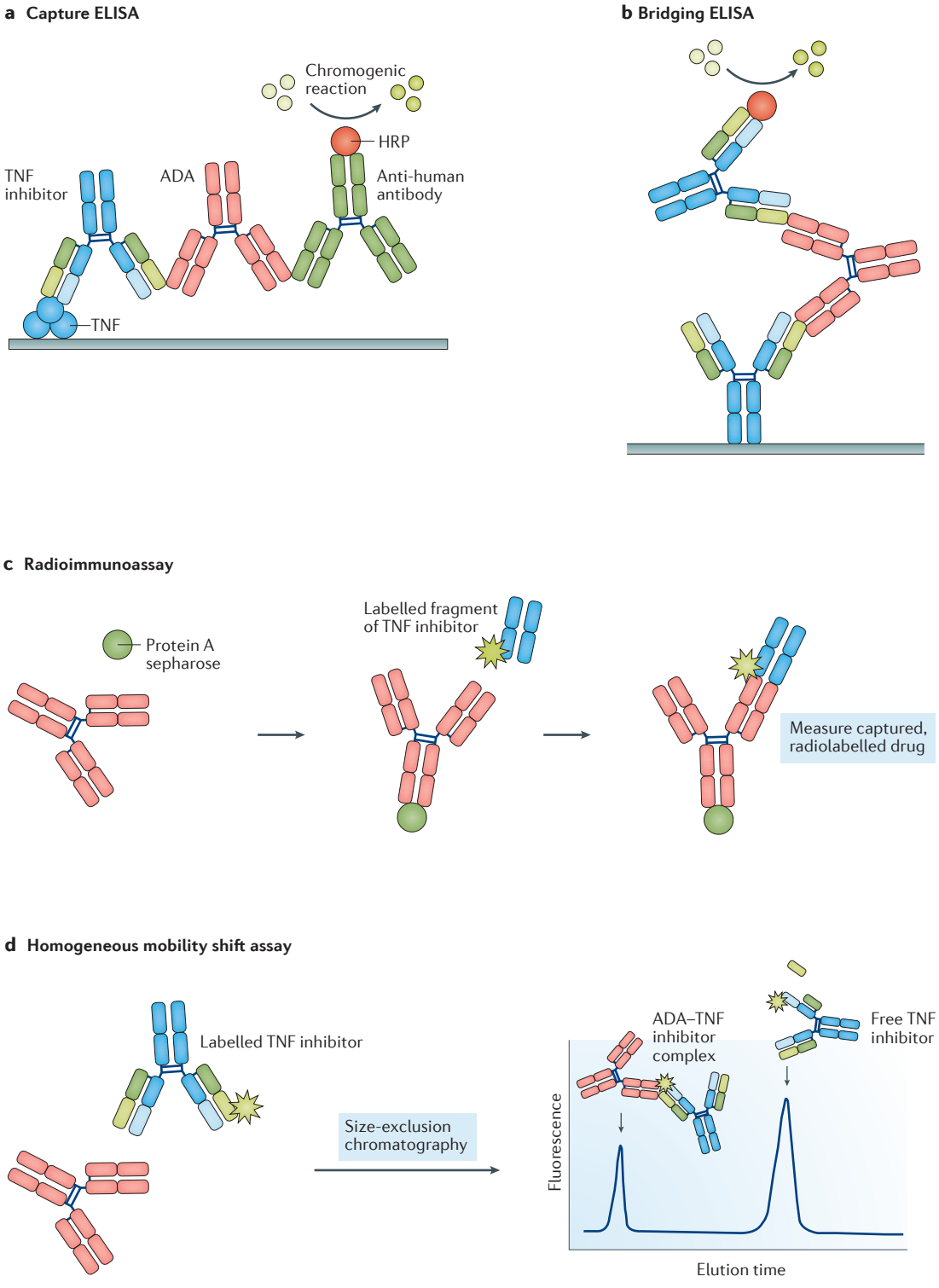

Figure 2 | **Assays for detecting antidrug antibodies to TNF inhibitors. a** | In a capture enzyme linked immunosorbent assay (ELISA), the TNF inhibitor is bound to TNF attached to the assay plate. Antidrug antibodies (ADAs) in the patient's serum bind to the drug, and are detected by horseradish peroxidase (HRP)-conjugated anti-human antibodies. **b** | In a bridging ELISA, the assay plate is coated with the TNF inhibitor, which in turn bind ADAs. ADAs are detected by use of HRP-conjugated drug. Bridging ELISA is susceptible to interference by the drug and typically measures ADAs only in the absence of detectable drug levels. **c** | In a radioimmunoassay, protein A sepharose captures ADAs in the patient's serum, which then bind to radiolabelled drug, and the amount of radiolabelled drug is then measured. Radioimmunoassay can capture clinically relevant IgG1 and IgG4 antibodies. **d** | A homogenous mobility shift assay uses size-exclusion chromatography to measure ADA–drug complexes.

Although future methods might overcome some of the discussed limitations in ADA testing, none of the test systems presently available can be accepted for routine clinical use[58–64]. These limitations seem to be responsible for the great variation reported with regard to the appearance of ADAs. Lastly, some data come from industry-supported clinical trials in which detailed information regarding the system used to test patient sera for the presence of ADAs is not given, which makes the interpretation of the available data even more difficult.

If ADAs are demonstrated, the question then arises of whether these antibodies could contribute to the loss of clinical efficacy of TNF antagonists by competing with TNF for the antigen-binding site. In this context, a study by van Schie *et al.*[64] demonstrated that in sera from 34 patients with RA treated with the anti-TNF antibodies adalimumab, golimumab or certolizumab pegol, more than 97% of ADAs were neutralizing antibodies. In 34 patients treated with infliximab, more than 90% of ADAs to infliximab were neutralizing[64]. Further characterization of the antibody response revealed that non-neutralizing antibodies to infliximab might bind infliximab-unique domains not involved in TNF binding. From these results it seems possible that, at least in some patients receiving TNF antagonists, the formation of ADAs might interfere with the efficacy of treatment.

Antibodies against TNF antagonists

Anti-infliximab antibodies

The frequency with which antibodies against infliximab can be detected in sera from infliximab-treated patients ranges from 7% to 53% (TABLE 1). The appearance of ADAs can be associated with reduced serum concentrations of (free) infliximab, in association with a decreased clinical response and with increased adverse events[65]. In RA, low serum concentrations of infliximab even 2 months after treatment initiation correlated with the formation of ADAs and predicted subsequent treatment failure[66]. Specifically in AS, several reports indicate that the formation of ADAs against infliximab is associated with undetectable trough serum levels of infliximab and with a reduced response to treatment, as well as with an increased risk of developing infusion reactions[66,67]. Together, these studies clearly indicate the

Table 1 | **Frequencies of antidrug antibodies to TNF inhibitors**

Disease	Patients (*n*)	Observation time (weeks)	Concomitant csDMARD	TNF inhibitor dose	ADA frequency	Refs
Infliximab						
RA	51	56	None	3 mg/kg	43%	65
	35	26	Methotrexate	3 mg/kg	51%	66
	43	26	Methotrexate	1 mg/kg	17%	80
				3 mg/kg	7%	80
				10 mg/kg	0%	80
	44	26	None	1 mg/kg	53%	80
				3 mg/kg	21%	80
				10 mg/kg	7%	80
	9	Up to 164	Methotrexate	3 mg/kg	33%	95
	8	Up to 164	None	3 mg/kg	50%	95
AS	38	24	None	5 mg/kg	18%	67
		54	None	5 mg/kg	29%	67
	8	24	None	5 mg/kg	25%	68
SpA	25	Up to 164	Methotrexate	5 mg/kg	0%	95
	66	Up to 164	None	5 mg/kg	21%	95
Adalimumab						
RA	271	24	Methotrexate	20 mg, 40 mg or 80 mg EOW	1%	8
	34	26	Methotrexate	40 mg EOW	29%	66
AS	35	26	None	40 mg EOW	31%	72
Etanercept						
RA	367	16	NR	50 mg per week	3%	9
AS	53	26	None	25 mg twice weekly	0%	76

ADA, antidrug antibody; AS, ankylosing spondylitis; csDMARD, conventional synthetic DMARD; EOW, every other week; NR, not reported; RA, rheumatoid arthritis; SpA, spondyloarthritis.

immunogenicity of infliximab on the one hand and the value of concomitant immunosuppressive therapy to suppress ADA formation in RA on the other.

Anti-adalimumab antibodies

Although adalimumab is a fully human monoclonal antibody, it still has immunogenicity. The frequency of the detection of anti-adalimumab antibodies in patients treated with adalimumab varies from 1% to 31% (TABLE 1), again most probably owing to the use of different assay systems. The antibodies against adalimumab are primarily anti-idiotypic antibodies, which elicit functional neutralization[68,69]. As with infliximab, trough levels of the therapeutic monoclonal antibody (adalimumab) are inversely associated with a loss of clinical efficacy[70–72]. It is worth mentioning that in these studies, adalimumab was given without concomitant immunosuppressive medication. Moving towards a personalized medicine approach, a 2013 study that included 221 patients with RA being treated with adalimumab demonstrated a relationship between adalimumab trough levels and clinical efficacy[73]. In this study, trough levels of 5–8 μg/ml were sufficient to elicit a clinical response. Importantly, these trough levels were substantially influenced by concomitant methotrexate medication[73].

Anti-etanercept antibodies

In a controlled clinical trial of patients with RA treated with etanercept, 3% of patients became ADA-positive (TABLE 1). Possible reasons for the low immunogenicity of etanercept have already been discussed (discussed above and reviewed elsewhere[48]).

Some data have been published relating to the correlation between trough levels of etanercept and clinical response: in particular, a correlation between trough drug levels and disease activity has been shown in AS as well as in RA[74,75]. By contrast, a separate study found no differences in etanercept serum trough levels in patients with AS who were classified as responders or non-responders[76]. This finding is somewhat surprising as it stands out from many other observations with different biologics, and the reason for this discrepancy remain unclear.

Antibodies against other anti-TNF agents

In a preliminary communication[77] it was demonstrated that in patients treated with golimumab over a 1-year period, those classified as responders had higher serum levels of golimumab compared with non-responders[77]. In three patients with high ADA titres, golimumab levels were not detectable and these patients had a poor clinical outcome. These results might indicate that with improved methodologies, the control of serum drug level and ADAs might also be useful for the follow-up of patients treated with golimumab[77].

ADAs against certolizumab have been reported in 3–25% of certolizumab-treated patients. A high incidence was found when patients with plaque psoriasis were repeatedly treated with certolizumab[78]. In patients with RA, the appearance of ADAs was associated with a modest reduction in therapeutic response and antibody responses were associated with low serum trough levels of the drug[79].

Treatment strategies

Concomitant immunosuppression

Foreign proteins including chimerized monoclonal antibodies are immunogenic and will elicit an immune response. In the case of human monoclonal antibodies, this response will be mainly be an anti-idiotypic response. Furthermore, it has been clearly shown that antibodies against TNF inhibitors (with the possible exception of etanercept) will have clinical implications, contributing to a loss of efficacy of TNF inhibitors. Despite a lack of availability of standardized assay systems for measuring ADA levels (FIG. 3), efforts have been made to prevent the formation of ADAs or to circumvent situations in which ADA occur. A study published in 1998 aimed to evaluate the efficacy, pharmacokinetics, immunogenicity and safety of multiple infusions of a chimeric monoclonal antibody to TNF (cA2, later renamed infliximab) given alone or in combination with low-dose methotrexate in patients with RA[80]. The combination treatment was well tolerated. When infliximab was given at a dose of 1 mg per kg bodyweight together with low-dose methotrexate, a synergistic effect with regard to controlling disease activity was observed. Although no test systems were available at that time for the demonstration of ADAs, the observed synergistic effect between the monoclonal antibody and methotrexate could possibly be explained by a sustained suppression of the formation of ADAs[80]. This publication marked the beginning of the combined use of TNF inhibitors and csDMARDs, mainly methotrexate, in the treatment of RA[81,82]. Methotrexate reduces immunogenicity in adalimumab-treated patients with RA in a dose-dependent manner[83]; consistent with this finding, data published in 2015 showed that increasing doses of methotrexate in combination with adalimumab improved clinical outcomes, mimicking the pharmacokinetic profile of adalimumab in patients with early RA[84].

Methotrexate significantly increases adalimumab trough concentrations, most likely via the suppression of ADAs[85]. Other csDMARDs such as leflunomide, hydroxychloroquine and sulfasalazine are also used in combination with a TNF inhibitor[86], although methotrexate seems to have the strongest influence on adalimumab trough levels[83]. Repeated reports of the superior clinical efficacy of a combination therapy that includes immunosuppressive agents versus biologic monotherapy led EULAR and the ACR to recommend that biologic DMARDs should always be used in combination with an immunosuppressive agent, primarily methotrexate, in the management of RA with biologic DMARDs[87,88].

The reasons for the described synergistic effect between methotrexate and biologics are not yet fully understood. The prevention of neutralizing ADAs will be part of this synergistic effect; furthermore, there could be an effect on the clearance of the biologic agent via the expression of Fc receptors on monocytes or a modulation of the interaction between the therapeutic antibody and Fc receptors[41,89,90].

Attempts to prevent the development of ADAs by patient-specific optimization of TNF inhibitor dosing have only been performed in a few situations.

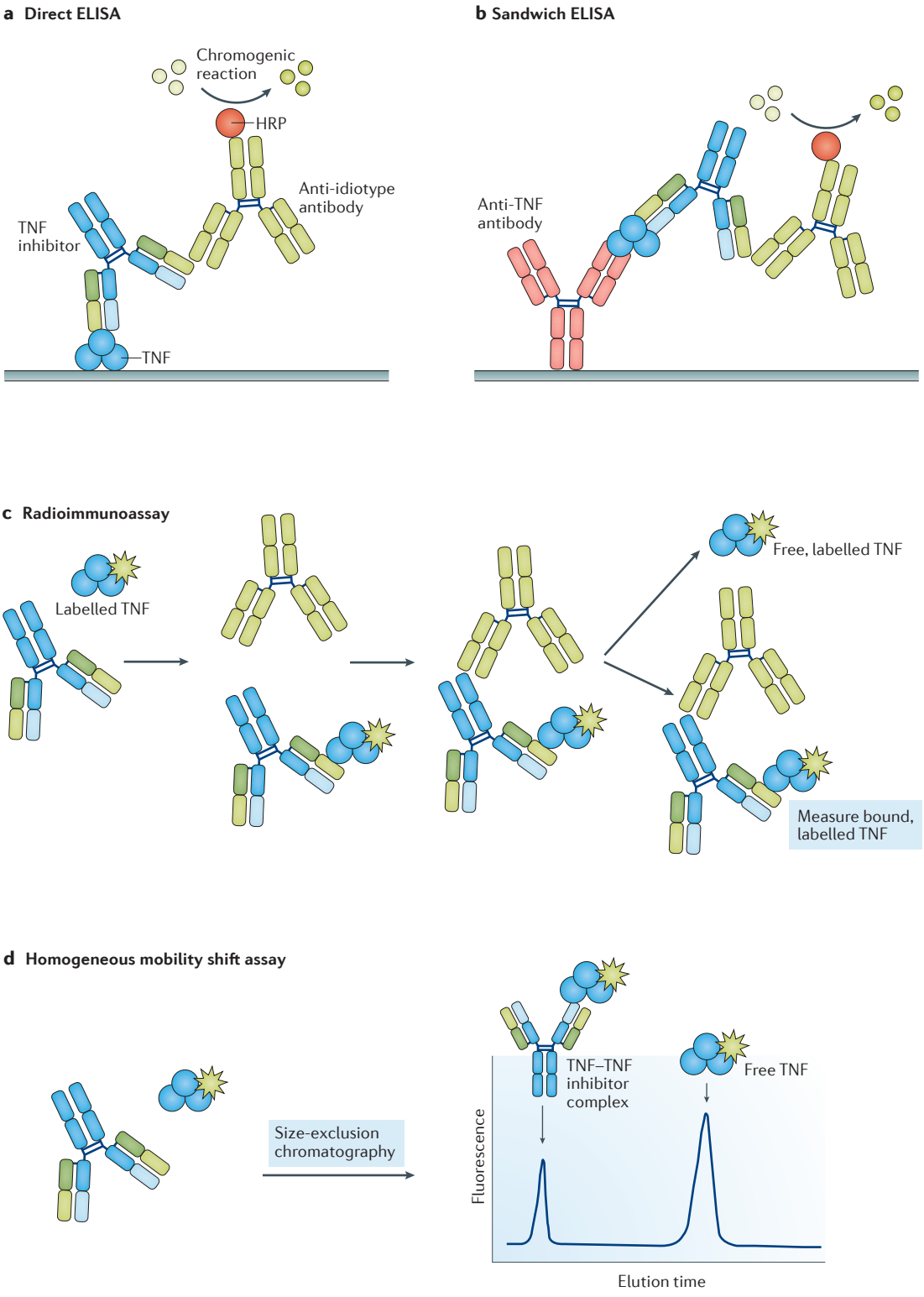

Figure 3 | **Methods to detect trough levels of therapeutic antibodies. a** | In a capture enzyme-linked immunosorbent assay (ELISA), the TNF inhibitor is bound to TNF attached to the assay plate. The drug is detected by horseradish peroxidase (HRP)-conjugated anti-idiotype antibodies. **b** | In a sandwich ELISA, the assay plate is coated with anti-TNF antibody bound to TNF. The TNF inhibitor binds to the TNF and is detected by use of HRP-conjugated anti-idiotype antibodies. **c** | In a radioimmunoassay, labelled TNF binds to TNF inhibitor in the patient's serum, which then binds to an anti-indiotype antibody. The amount of radioactive drug is measured. **d** | A homogenous mobility shift assay uses size-exclusion chromatography to measure antibody–drug complexes.

In Crohn's disease[91], different infliximab dose-optimization strategies have been discussed, such as the use of induction doses at 0, 2 and 6 weeks, followed by a systemic maintenance therapy administered every 8 weeks. This strategy protected against ADA formation in Crohn's disease in a manner that might not require concomitant immunosuppressive medication. In addition, this regimen seems to provide superior disease activity control with a lower relapse rate over 1 year[92–94]. The effectiveness of this three-dose treatment regimen in Crohn's disease has not been verified in a randomized controlled trial and, furthermore, one has to take into consideration that this type of treatment might lead to a different clinical outcome in RA. In patients with RA treated with 3 mg per kg infliximab intravenously at weeks 0, 2, 6 and 14, and every 8 weeks thereafter, it was obvious that higher trough levels of infliximab during treatment initiation reduced the development of ADAs and were associated with a prolonged maintenance of infliximab[95]. Similarly, in patients with Crohn's disease, particularly in the group of patients receiving 10 mg per kg bodyweight of infliximab, those on a scheduled treatment strategy reported better disease activity outcomes than those who received episodic treatment[96]. This finding is reminiscent of the high-dose tolerance induced by a high antigenic load as originally proposed by Avrion Mitchison[97].

The idea that the prevention of ADAs by co-medication with methotrexate in patients treated with TNF antagonists is not the final solution to prevent ADA formation comes from a 2014 study in psoriatic arthritis. Here, it was demonstrated that patients with psoriatic arthritis experienced a similar TNF inhibitor persistence on combination therapy (that is, with methotrexate) as they did on monotherapy (that is, TNF inhibitor only, without concomitant methotrexate)[98].

Dealing with antidrug antibodies

In patients with ADAs and loss of clinical efficacy of a TNF inhibitor, the possibility exists to switch to another anti-TNF molecule or to another biologic, including humanized antibody molecules, that might be less immunogenic. The success of switching to a second TNF inhibitor has been demonstrated in a number of studies, including those from national registries[99–105]. Switching to a second TNF inhibitor might work for several reasons, including differences in the molecular (protein-based) structure of the biologics, immunological action, immunogenicity and different pharmacokinetics[21,41,42]. Different pathogenic pathways that underlie diseases might also determine the success of this switching practice[106]. Published data also suggest that after discontinuation of an initial TNF inhibitor, switching to rituximab or to another biologic might be associated with improved clinical effectiveness compared with switching to a second TNF inhibitor[107–109].

Future directions

To date, there is no way to distinguish a non-responder from a responder before commencing treatment with a biologic in combination with a csDMARD. Likewise, no biomarkers are available that will tell the treating physician if the patient will need to discontinue treatment with a TNF inhibitor or not. Several easily characterized clinical variables are associated with risk of discontinuation, including physician global assessment, disease activity and the number of TNF inhibitors used previously[109]. Hopefully, in the near future, methods will become available that will help to separate responders from non-responders to a given biologic before treatment is started and, in addition, parameters related to the outcome of a patient receiving biologic medication will be defined.

As discussed, at the moment there is no standardized test system available for the detection of ADAs. The same seems to be true for the measurement of drug trough levels. Therefore, estimation of ADAs and/or serum trough levels in routine patient care does not seem feasible. Testing for ADAs might be indicated in a situation in which a patient is no longer responding to a given monoclonal therapeutic agent. The same might be true in the future for bispecific antibodies. With regard to fusion proteins such as etanercept, the estimation of serum trough levels might be indicated in a situation in which there is a loss of clinical efficacy. To at least reduce the development of ADAs, one should follow the recommendations put forward by EULAR and ACR to use biologic agents (including tocilizumab) in combination with a csDMARD, preferentially methotrexate.

In situations in which ADAs already exist, switching to another TNF inhibitor or to another biologic is indicated. Patients who fail to respond to infliximab or to other anti-TNF monoclonal antibodies should be switched to the B-cell-depleting agent rituximab. When patients are switched from treatment with infliximab to adalimumab because of ADAs, one has to take into consideration that those with anti-infliximab antibodies are prone to develop *de novo* anti-adalimumab antibodies and that these new antibodies might result in the therapeutic failure of adalimumab[110]. Similarly, if patients with ADAs to a particular TNF inhibitor are switched to a biosimilar, ADAs to the originator product will cross-react with the biosimilar, as shown in patients treated with Remsima or Inflectra who had developed ADAs to infliximab[111,112].

Should standardized methods become available in the future, measuring serum trough levels of biologics seems to be a fair possibility in the follow-up of patients treated with biologics. Trough drug levels inversely mirror ADA formation and, furthermore, trough levels of fusion proteins can be inversely associated with disease activity[74]. Finally, as discussed for adalimumab, trough drug levels might be used in the future to estimate the specific trough level for a certain patient with RA and thus could help to find the best dose of a biologic for that patient[113]. Monitoring drug levels might also help clinicians to optimize the dosing regime and prevent overtreatment for patients receiving anti-TNF therapy[114], and might predict (at least in Crohn's disease) infliximab failure during maintenance therapy[115,116].

Conclusions

The introduction of monoclonal antibodies and fusion proteins to block TNF in patients with RA was an important milestone in our treatment options for these diseases. However, as foreign proteins, the various anti-TNF drugs might induce ADAs, with differences in the occurrence and magnitude depending on the treatment regimen.

ADAs have effects on drug efficacy and safety, which might be explained in part by the conformation of the drug itself, the use of concomitant immunosuppressive medication and differences in dosing regimens. More importantly, ADAs have a major role in causing a secondary response failure, in which effectiveness of a TNF inhibitor is lost over time despite a good initial response.

There is currently no consensus regarding the definition of primary and secondary drug failure. Likewise, there are currently no evidence-based recommendations to guide biologic therapy on the basis of drug levels and immunogenicity testing. In addition, problems with the detection of ADAs and/or the measurement of serum trough drug levels, as well as the clinical observation that not all ADAs are neutralizing, have prevented the routine monitoring of patients receiving anti-TNF treatment. Faced with a patient's failure to respond to a given TNF antagonist, many treating physicians will switch to another TNF antagonist or to a biologic with a different target.

In our view, there are presently few situations in which tests for ADAs and serum trough levels are indicated. Estimation of serum trough drug levels will be important in patients who have primary failure to respond to an anti-TNF drug. Thus, if the serum trough level is low and no ADAs are present, an increase in dose or a shortening of the interval between doses might lead to a clinical improvement. A similar procedure can be recommended in patients with secondary treatment failure: if the clinical situation does not improve, then treatment should be switched to another TNF antagonist or to a different biologic. With biosimilars, one has to be aware that ADAs against the originator drug will crossreact with the respective biosimilar, thus possibly leading to further treatment failure.

With more data on the immunogenicity of anti-TNF drugs and the availability of better test systems, both for ADAs and trough levels, a strategy of immune-pharmacologic guidance to individualize treatment of patients with RA might become feasible in the near future and possibly reduce treatment costs[117].

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Native joint-resident mesenchymal stem cells for cartilage repair in osteoarthritis

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Abstract | The role of native (not culture-expanded) joint-resident mesenchymal stem cells (MSCs) in the repair of joint damage in osteoarthritis (OA) is poorly understood. MSCs differ from bone marrow-residing haematopoietic stem cells in that they are present in multiple niches in the joint, including subchondral bone, cartilage, synovial fluid, synovium and adipose tissue. Research in experimental models suggests that the migration of MSCs adjacent to the joint cavity is crucial for chonodrogenesis during embryogenesis, and also shows that synovium-derived MSCs might be the primary drivers of cartilage repair in adulthood. In this Review, the available data is synthesized to produce a proposed model in which joint-resident MSCs with access to superficial cartilage are key cells in adult cartilage repair and represent important targets for manipulation in 'chondrogenic' OA, especially in the context of biomechanical correction of joints in early disease. Growing evidence links the expression of CD271, a nerve growth factor (NGF) receptor by native bone marrow-resident MSCs to a wider role for neurotrophins in OA pathobiology, the implications of which require exploration since anti-NGF therapy might worsen OA. Recognizing that joint-resident MSCs are comparatively abundant *in vivo* and occupy multiple niches will enable the optimization of single-stage therapeutic interventions for OA.

'Chondrogenic' OA
A type of osteoarthritis (OA) in which early lesions form in the articular cartilage; distinct from OA that starts in other structures, such as OA that begins following meniscus or bone injury.

The pathogenesis of osteoarthritis (OA) is complex and heterogeneous, with both disease initiation and progression being dependent on multiple joint structures, including cartilage, bone, ligaments, meniscus and synovium[1,2]. Many research articles and reviews have emphasized the role of culture-expanded cellular therapies, scaffolds and drugs in the development of therapies for OA, especially for 'chondrogenic' OA, but there is a paucity of data on the use of native (not culture-expanded) joint-resident stem cells in joint-repair strategies. This Review will focus on 'chondrogenic' OA, in which disease initiation and progression seem to be critically dependent on the articular cartilage. The role of subchondral bone, including the osteochondral junction, is also important in the pathogenesis of OA and has been discussed extensively elsewhere[3]; therefore, our comments on this subject will largely focus to the role of native bone marrow-resident stem cells, especially at sites of cartilage denudation in advanced OA, where such topographically localized cells can directly access the joint cavity.

The pivotal role of articular cartilage loss in OA[4,5] and the recognition that cartilage can be restored, albeit with relatively poor-quality repair tissue, following microfracture techniques in patients with isolated cartilage lesions or following autologous chondrocyte implantation for the treatment of full-thickness lesions[6,7], pointed to the potential importance of cartilage in the development of therapies for OA. These early studies[6,7] suggested that cartilage repair could occur via the actions of highly proliferative cells in close proximity to the cartilage, and were a key impetus for the subsequent culture expansion cellular protocols (first popularised in the 1990s[6]) and for the subsequent joint-repair strategies that used combinations of culture-expanded cells and adjuncts, including scaffolds and pharmaceutical agents[8]. Although it might not be possible to extrapolate the potential benefits of cellular therapy from results in isolated cartilage defects in young individuals to defects in patients with advanced OA, there is evidence that isolated cartilage lesions in skeletally mature individuals increase in severity over time[9–11], suggesting that advances in the treatment of early lesions could help to prevent OA in later life.

Previously, despite spontaneous articular cartilage regeneration being considered unlikely, seemingly misguided reparative responses (in the form of chondro-osteophyte formation) were recognized to occur. In the past few years, the spontaneous repair of full-thickness cartilage defects was noted in humans following joint

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Key Points

- Although historically considered to be very rare cells, native mesenchymal stem cells (MSCs) are actually relatively abundant *in vivo*
- Joint-resident MSCs occupy several bone and joint cavity niches including synovium, adipose tissue and synovial fluid
- Advanced osteoarthritis (OA) is associated with a numerical increase, but functional decline, in MSCs in regions of MRI-determined bone oedema, suggesting direct involvement of MSCs in OA pathology *in vivo*
- The expression of CD271 (also known as low-affinity nerve growth factor receptor) on native bone marrow-resident MSCs might be important in pathological bone changes following anti-nerve growth factor therapy
- In experimental models, there is strong evidence for the involvement of synovium-derived MSCs in cartilage repair following joint injury
- Emerging features of joint-resident MSCs suggests the potential for their use in the development of single-stage therapy to treat large cartilage defects in patients with OA

offloading, either by re-alignment osteotomy[12] or by total joint distraction techniques[13,14], with neither procedure directly breaching the joint cavity. These reparative events did not depend on cell expansion protocols but instead harnessed native joint-resident or periarticular cells in a manner reminiscent of early microfracture methodologies, which also harnessed endogenous reparative capabilities[7]. Importantly, these procedures highlighted the fact that the addition of scaffolds or growth factors was not essential for endogenous repair in chondrogenic OA[15]. As such, in this Review we largely confine our comments to the emerging evidence for a cellular basis for regenerative mechanisms in OA, and focus on cartilage repair.

At the cellular level, spontaneous cartilage regeneration suggests potentially overlapping roles for stem cells from different niches and also for mature chondrocytes (FIG. 1). In this Review, we focus on a subgroup of adult stromal cells that are highly proliferative, clonogenic and capable of multi-lineage differentiation into mesenchymal tissues including bone, cartilage and adipose tissue. As such, these cells are referred to as mesenchymal stem cells (MSCs), alternatively known as mesenchymal stromal cells or marrow stromal cells (when originating from trabecular bone), all of which bear the MSC acronym.

Mesenchymal stem cells

The high proliferative capacity of cultured MSCs and their chondrogenic capabilities have catapulted them to the forefront of cellular therapy development for OA. A large body of literature has accrued on culture-expanded MSCs, which are being trialled as a therapy for OA[16,17], but the combination of expense and limited long-term efficacy still presents a major hurdle to the adoption of this therapy. To make such procedures single-stage, there is interest in using 'off the shelf' allogeneic MSCs. Although allogeneic MSCs might have immunomodulatory effects, they are also associated with potential problems, including loss of functionality following *in vitro* expansion and culture-induced senescence[18]. The culturing of manipulated cells will not be discussed further in this Review as artificially aged *in vitro* cellular therapies might not function efficiently in the hostile environment of the osteoarthritic joint[19].

Understanding of the role of MSCs in OA has been influenced by historical misconceptions about MSCs, which originated from our knowledge of haematology. In the haematopoietic stem cell (HSC) model, a single HSC can repopulate the entire haematopoietic system[20]. Like the HSC, the MSC was also viewed as a rare, highly proliferative, clonogenic, multipotent cell that could circulate systemically to reach remote sites[21]. In hindsight, the shared origin of HSCs and bone marrow-resident MSCs might have resulted in the idea that stem cell progeny can leave the marrow cavity to home to distant sites. In reality, however, apart from being co-housed in the skeleton, both systems are radically different; for example, HSCs are rare, quiescent progenitors that reside in a specific niche, whereas cells with MSC-like characteristics can be readily derived *in vitro* from abundant mature stromal cells, including chondrocytes[22] and adipocytes[23] (FIG. 1). This evidence supports the idea that fully differentiated somatic cells such as chondrocytes might contribute to tissue repair without the need for differentiation from MSCs or some intermediate cell, and challenges traditional stem cell concepts. Moreover, joint-resident cells with a fibroblastic morphology and features of MSCs can occupy multiple tissue niches (FIG. 1). Unlike the HSC model, it is difficult to comprehend how a single MSC could recapitulate the entire skeletal system, and the derivation of an animal model along the same lines as the HSC model is highly improbable.

Bone marrow-resident MSCs. Compared with extraosseous MSCs, our understanding of the biology of bone marrow-resident MSCs is more advanced in terms of phenotype, topography, function and potential therapeutic applications. The bone marrow compartment has an important role in advanced OA and MRI-determined bone marrow oedema is prognostically relevant[5]. Moreover, the theory behind the 'original' stem cell therapy (using microfracture to treat isolated cartilage defects that are thought to be associated with the development of OA[10,11]) was predicated on the idea that bone marrow-resident MSCs percolate through to the cartilage from the bone marrow and act as the cellular building blocks for tissue repair[24]. At birth, the articular cartilage might be indistinguishable from the epiphyseal growth plate in both humans and mice as a result of the secondary epiphyseal cartilage ossification centres having not yet formed[25]. However, since the focus of this Review is on MSCs in adult joint repair, the intricate links between articular cartilage and the adjacent cartilage of the epiphyseal plate destined to become subchondral bone will only be briefly touched upon with regards to the mechanism of cartilage growth during development and during the neonatal period.

Bone marrow-resident MSCs coexist with HSCs, with both cell types able to exert homeostatic control over each other's functions[26]. This unique microenvironment seems to have a profound effect on the physiological demands on MSCs: not only do bone marrow-resident MSCs control host tissue remodelling, homeostasis of adipose tissue in bone and bone repair following fracture, but they also support HSC function, maturation and circulatory egress[27].

Osteotomy
A technique whereby bone is surgically realigned to change the joint alignment and load distribution.

Total joint distraction
A surgical technique in which external fixator devices are placed across the joint to restore the joint space; associated with cartilage repair.

Epiphyseal cartilage ossification centres
Areas of the cartilagenous growth plate at the metaphyseal ends of long bones in which bone formation follows the primary ossification seen in the diaphysis of long bones.

In other words, bone marrow-resident MSCs can be considered more 'multi-functional' than MSCs in tissues in which HSC support is not a physiological requirement, such as other tissues of the joint. Moreover, native bone marrow-resident MSCs are part of the adventitial reticular compartment (also known as the stromal marrow supportive cellular compartment), which is a functionally mature and abundant cell population[28–30].

The original isolation and characterization of bone marrow-resident MSCs was based on the ability of rare bone marrow-derived cells to adhere to plastic and proliferate *in vitro* to form fibroblastic colonies[31]. In humans, native bone marrow-resident MSCs are characterized by being negative for the expression of haematopoietic cell and endothelial cell markers (for example, CD45 and CD31) and being positive for several other markers such as CD90 and CD73. The most commonly used maker for native bone marrow-resident MSCs is CD271 (also known as TNF receptor superfamily member 16, low-affinity nerve growth factor receptor (LNGFR) or p75 receptor)[32]. Data from the past few years suggest that bone marrow-resident MSCs have diverse embryonic origins (being both neural crest-derived and mesoderm-derived[33]) and that the relative proportions of cells from each origin might depend on several factors, including bone type and stage of development (for example, whether the bone is from a neonate, a child or an adult)[34,35]. In mouse models, Gremlin 1$^+$ progenitor cells, dubbed osteochondroreticular cells, participate in bone repair[35]. In our opinion, the osteochondroreticular cell population represents a more primitive population of MSCs than those carried into the limb bud during the development of the bone marrow niche, with the latter type of MSCs imbued with both tissue regenerative and haematopoietic support capabilities[36]. Although osteochondroreticular cells contribute to fracture repair in murine models, the role of this population in cartilage repair in OA remains conjectural[35].

Bone marrow-resident MSCs are the only type of MSC for which a capacity for self-renewal, in the context of relevant host tissue regeneration, has been demonstrated *in vivo* at the single-cell level[37,38]. A single culture-expanded bone marrow-derived MSC can regenerate a whole ectopic bone organ (termed the bone ossicle), containing not only newly formed bone, but also haematopoiesis-supporting stroma that can later be repopulated by host HSCs[37]. Even though this bone ossicle assay has limitations (such as an inability to recapitulate native mechanical demands on the formed bone or to be used for testing

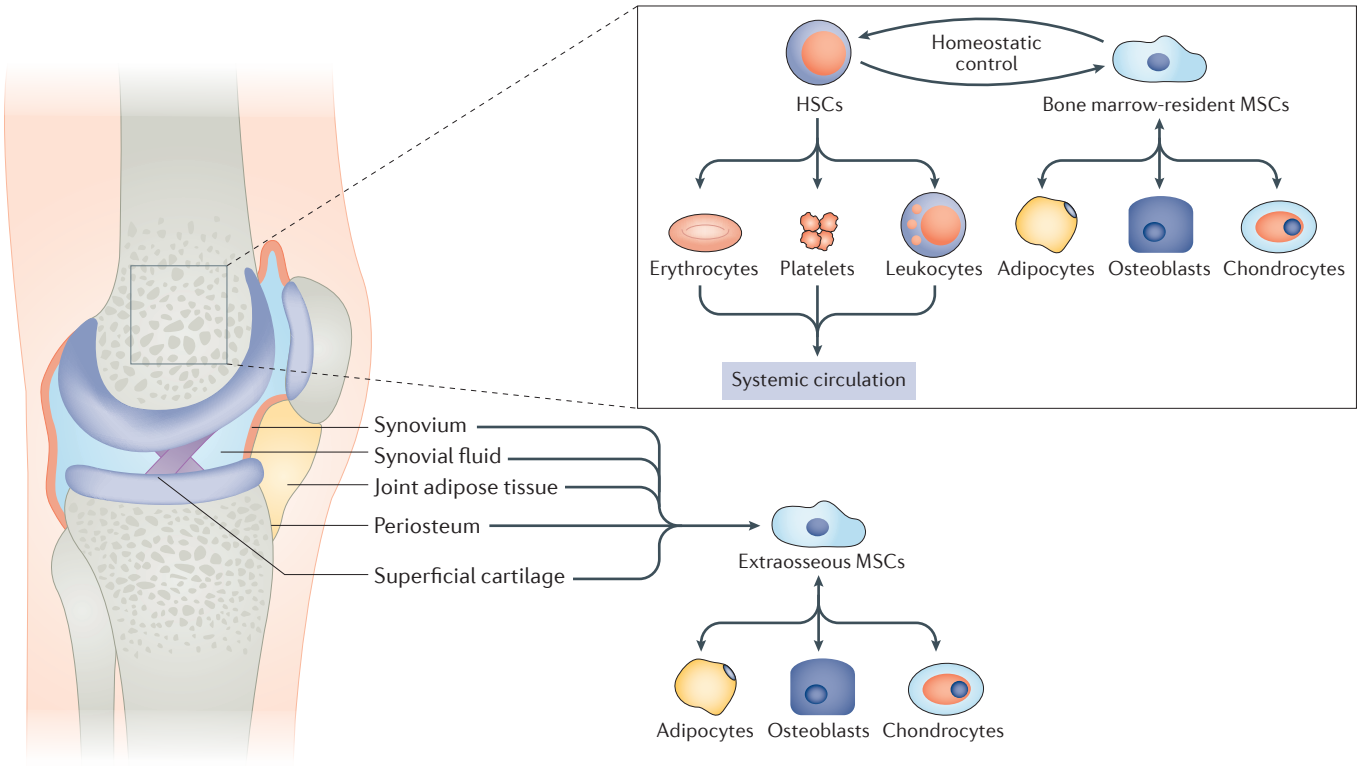

Figure 1 | **Stem cells in the joint.** The hypothesis that mesenchymal stem cells (MSCs) need to access the circulatory system to reach their destination was adapted from the haematopoetic stem cell (HSC) model. Both MSCs and HSCs are found in the bone marrow, but MSCs have also been described in multiple other niches within the joint, including the periosteum, synovium, adipose tissue (for example, the infrapatellar fat pad) and synovial fluid, as well as other periarticular tissues. Given the avascular nature and considerable thickness of some types of cartilage, a model in which multiple local populations of extraosseous MSCs exist with ready access to superficial zone cartilage is superior to the HSC model for the direct repair of cartilage lesions (without the need for systemic circulation or long-range migration of MSCs from the bone marrow). For MSCs, there is ample evidence that mature mesenchymal lineage cells such as adipocytes and chondrocytes can 'dedifferentiate' (indicated by arrows from daughter cell to MSC) into MSCs and exhibit high proliferative capacity and multipotentiality. The recognition that fully differentiated stromal cells can readily adopt MSC-like characteristics following *in vitro* adhesion to plastic argues against the feasibility of discovering specific MSC markers in synovium, cartilage or other joint tissues.

single, purified, non-culture expanded MSCs), it is the gold standard assay for showing the 'stem cell nature' of bone marrow-derived MSCs. However, the results of such elaborate assays are difficult to translate into the site-specific need for chondrocytes, osteoblasts and osteocytes in OA-related cartilage repair, especially in the context of joint-resident MSCs in early OA, which do not need to provide a supportive role to HSCs.

As previously mentioned, bone marrow-resident MSCs occupy the perivascular niche, as well as the stromal reticular niche[29], where they are known as adventitial reticular cells[39] and form a cellular net, laying down extracellular matrix to support and anchor other cells of the bone marrow. Bone marrow-resident MSCs are thought to be found at the inner surfaces of the bone[40], enabling them to participate directly in bone remodelling processes without the need to migrate to perivascular or reticular locations. Importantly, adult bone marrow-resident MSCs do not follow a chondrogenic differentiation programme unless bone fracture occurs, triggering the endochondral ossification pathway, whereby a provisional cartilaginous tissue (soft callus) is initially formed[41]. In addition, as previously mentioned, bone marrow-resident MSCs are capable of supporting homeostatic, as well as on-demand 'emergency' haematopoiesis[42], the latter property being unique to bone marrow-resident MSCs and not physiologically relevant for joint-resident MSCs required for joint repair strategies.

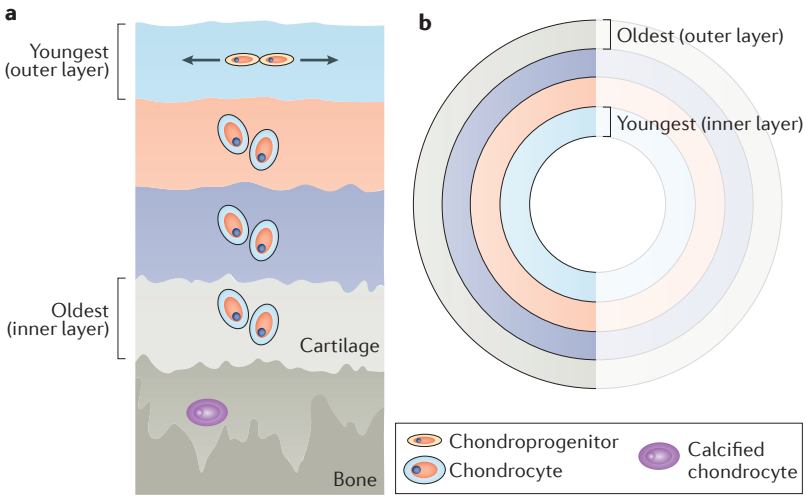

Figure 2 | **Appositional growth versus interstitial growth. a** | For appositional growth, resident chondroprogenitor cells within superficial zone cartilage divide and generate the underlying layers and cartilage matrix. During development, the cells in the superficial layer 'stack up' and the endochondral bone formation that occurs during epiphyseal plate growth results in cells that originated in the superficial zone eventually appearing in the bone matrix. The chondroprogenitor cells are responsible for maintaining the superficial zone progenitor population and might exhibit lateral migration; daughter cells reside in the older, innermost zones found deep within the cartilage, with the outermost zone being the youngest. Mesenchymal stem cells originating from the joint cavity, including the synovial fluid and synovium, can access superficial zone cartilage and complement the actions of these resident chondroprogenitor cells. **b** | In full-thickness cartilage defects that penetrate the underlying trabecular bone, bone marrow-resident mesenchymal stem cells can become exposed. In this setting, interstitial growth or hyperplasia of bone marrow-resident MSC-derived provisional tissue occurs.

Native bone marrow-resident MSCs in OA. The results of bone marrow aspirations showed that native bone marrow-resident MSCs were extremely rare in elderly individuals compared with young individuals, and that these age-related changes mirrored the distribution of age-related diseases including OA and osteoporosis[43]. Knowledge of the *in vivo* phenotype of bone marrow-resident MSCs and the recognition that bone marrow-resident MSCs colocalize with adventitial reticular cells led to the realization that such MSCs might not necessarily be released from the marrow during aspiration[29,44]. More suitable bone marrow digestion protocols were subsequently developed, the results of which indicated that the frequency of $CD271^+$ bone marrow-resident MSCs is in the order of ~1%[30]. This result challenged the concept that bone marrow-resident MSCs are so rare that expansion *in vitro* is necessary before they can be used therapeutically. Indeed, it is now possible to procure good manufacturing practice (GMP)-quality uncultured bone marrow-derived MSCs for orthopaedic applications without resorting to *in vitro* manipulation to bolster MSC numbers, although this technique has yet to be full exploited in humans[45].

Notably, CD271, the most robust surface molecule used for the isolation of bone marrow-resident MSCs, is a nerve growth factor (NGF) receptor[32,46]. The link between angiogenesis and pain in OA is well-established[47], but what is less well-appreciated is the fact that CD271 is typically expressed on bone marrow-resident MSCs in the perivascular niche[40]. Thus, MSCs might not only provide vascular support, but merit consideration as cells that could influence the association of perivascular neuronal ingrowths with osteoarthritic tissue[48,49]. In fact, data from a 2017 study has now shown how mechanical loading can induce the expression of NGF in osteoblasts, resulting in the activation of high-affinity NGF receptor-positive sensory neurons that provide osteogenic cues and facilitate increases in bone mass[50]. These data suggests a clear link between mechanical load, pain pathways and bone formation, which could be a factor in subchondral sclerosis in patients with OA.

Cartilage-resident MSC-like progenitor cells. Our understanding of the biology of cartilage-resident stem cells in health mostly comes from data from animal models, so great care is needed in extrapolating the relevance of this knowledge to patients with OA. The idea that articular cartilage is merely a remnant of epiphyseal cartilage that resisted the advancing front of endochondral ossification and contains residual stem cells is now obsolete, and the associated idea that cartilage regeneration or turnover starts in the deep zone of cartilage seems disadvantageous, as damage typically manifests in the superficial zone in early chondrogenic OA[51]. Studies by the Archer group into the morphology of the mammalian joint during neonatal and post-partum development in which chondrocytes were labelled with intra-articular bromodeoxyuridine indicated that chondrocytes were likely to be replenished from the superficial zone (termed appositional growth), rather than from the deep zone (interstitial growth)[52] (FIG. 2). Subsequent *in vitro* studies from the same group

confirmed the presence of an MSC-like resident population in the superficial zone[53]. Independent studies demonstrated that during growth, the deep regions of cartilage that are present at the end of the bones at birth are replaced by bone concurrently with the neoformation of articular cartilage adjacent to the joint cavity[54]. Indeed, only the superficial zone cartilage was left unaffected by the remodelling process: following remodelling, the superficial zone contained a cell population that exhibited bidirectional mitotic activity (either horizontal or vertically) and that replenished the pool of cartilage cells by lateral and vertical expansion of the tissue[54].

Elegant cell-fate mapping studies in mice that exploited the fact that embryonic joint interzone cells and superficial chondrocytes both express *Prg4* (encoding lubricin) have confirmed the importance of superficial zone cartilage in tissue homeostasis[55]. Superficial zone cells in young mice served as progenitors for both superficial zone and deep zone chondrocytes in older mice, and not only did the expansion of such cells fill deep zone cartilage with cells, but daughter cells were also found in the underlying subchondral bone of mature animals[55]. Although epiphyseal growth plate chondrocytes and articular chondrocytes arise from distinct progenitor populations, the discovery of daughter cells from superficial zone cartilage in bone validates previous observations of deep zone chondrocytes being able to form bone[54]. In 2017, two subpopulations of cartilage-resident MSC-like progenitor cells were identified in cartilage from patients with OA, one of which exhibited an early senescent phenotype, which possibly reflects a replicative exhaustion following repeated but failed attempts at cartilage repair[56]. The *in vivo* phenotype of these cells has not been defined, but the findings are reminiscent of the loss of proliferative capacity in $CD271^+$ MSCs found in the bone of patients with OA[49]. Together, these studies[49,56] indicate that MSC senescence and an associated loss of potency could be an important facet of OA pathophysiology. The possibility of migrating interstitial cells contributing to chondrocyte clustering has also been noted[57] and tallies well with the aforementioned study[54] that shows how horizontal and vertical cell migration ultimately originates from the superficial zone, providing a mechanistic connection between the superficial zone and repair in deep cartilage regions[55].

Superficial zone chondrocytes express α-smooth muscle actin at greater levels than deep zone chondrocytes, which supports the idea of enhanced migratory activity at sites of fissuring and fibrillation[58]. Notably, bone marrow-resident MSCs also express α-smooth muscle actin *in vivo*[59], but the absence of a robust marker for MSCs in cartilage has hampered a better understanding of the role of putative cartilage-resident MSCs in cartilage repair. In addition, culture-expanded cells derived from the cartilage of patients with OA (of which the precise topographic origin is unknown) are capable of undergoing long-range migration of >1 mm *in vitro*[60]. In the following sections, we consider the evidence that the superficial zone cartilage-resident cells are derived in turn from MSCs that originate in the joint cavity.

Joint-resident MSCs

Spontaneous chondrogenesis (chondromatosis) in the synovium is a well-recognized phenomenon in humans. In an adaptation to high local levels of tissue compression within the joints, articular fibrocartilage lines the surface of bones in elaborate structures (termed synovio-entheseal complexes) at sites where ligaments or tendons compress the adjacent bone[61], and in animal models the implantation of cartilage mitogens in or adjacent to the synovium triggers local synovial chondrogenesis[62]. Collectively, these observations indicate that the joint environment is poised to support chondrogenesis in locations beyond the classically defined opposing articular cartilage surfaces. The native cells responsible for this remarkable synovial chondrogenesis in humans have not yet been identified, so in the following section we discuss potential candidate cells.

Unlike the bone marrow microenvironment (in which all MSCs express CD271[63]), whether all joint-resident (including synovial fluid-resident) MSCs express CD271 is less clear. A unique set of markers that can select the entire highly proliferative multipotent stromal fraction isolated from the synovium and joint adipose tissue has not been universally agreed on; however, some studies have reported markers that can be used to select a distinct MSC subset with high chondro-osteogenic potency from culture-expanded, but not from freshly isolated, synovial cell populations[64]. At present, we would summarize that the literature shows the presence of a phenotypically heterogeneous stromal fraction in joint tissues that exhibit MSC-like activity, and it is therefore rather difficult to make definitive statements about the specific phenotypes of joint-resident MSC populations and their contributions to cartilage repair.

Synovial-resident and joint adipose tissue-resident MSCs. In mice, cells expressing growth/differentiation factor 5 (GDF5) give rise to articular cartilage, ligaments and the inner synovial lining (FIG. 3a), but hardly any contribution has been attributed to these cells in the formation of adjacent long-bone cartilaginous shafts or growth plates, indicating a very close embryologic link between the cartilage and the synovium[55,65]. Joint cavity-related MSCs were first reported in the synovium[66], but it is still unclear whether these cells originate from the superficial synovial lining or are of subsynovial origin, or both. Indeed, the synovium is a potent and rich source of chondrogenic MSCs, which are found at a frequency of ~1%[67,68], a similar frequency to that estimated following bone marrow digestion protocols[30,44] and a far greater frequency than that found in bone marrow aspirates[54].

In rabbit models, the synovium covers the superficial cartilage, and this synovial membrane contributes to cartilage repair[69]. Given the large size of human knee joints, it is unlikely that superficial synovium-resident MSC-like chondroprogenitor cells are able to migrate over the long distances required to reach the site of action. In one mouse model of cartilage injury, spontaneous cartilage repair did not occur *in vivo* but chondrogenic activity was evident at the joint margin[70]. The same study indicated that putative *in vivo* $CD271^+CD44^+$ MSCs

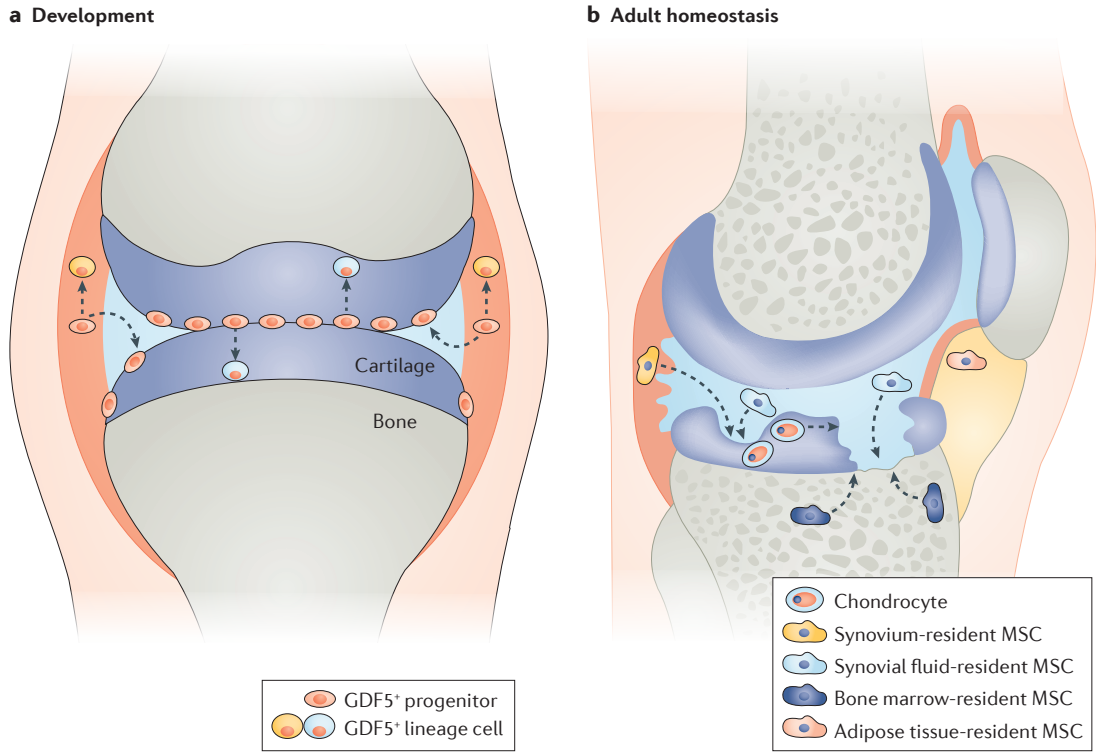

Figure 3 | **Progenitor cells in joint development and cartilage repair. a** | During development, growth/differentiation factor 5 (GDF5)-expressing progenitor cells are responsible for the initial joint cavitation. This population of cells can continue to reach the cartilage surface via periarticular tissues such as the synovium. In early development, appositional growth of cartilage is driven by superficial zone GDF5$^+$ cells. **b** | In adults, it is unclear how bone-marrow resident mesenchymal stem cells (MSCs) could contribute to the repair of superficial cartilage injuries. However, maintenance and repair of superficial (as opposed to full-thickness) injuries could involve resident cartilage progenitor cells or the migration of synovial fluid-resident and synovium-resident MSCs[83]. Indeed, direct migration of MSCs from the synovium and synovial fluid to sites of superficial cartilage injury has been shown experimentally, as has the contribution of synovial fluid-resident MSCs to ligament and meniscus repair[81–83]. Mature chondrocytes from within the cartilage are also capable of proliferation and migration. Lateral migration of adjacent cartilage-resident cells, including chondroprogenitors and chondrocytes, might also have a role in such settings, together with the migration of MSCs from the synovial fluid. Direct migration of native MSCs from the periarticular margins is also possible. For full-thickness cartilage loss, especially with breach of the subchondral plate, bone marrow-derived MSCs might contribute to the repair process from the osseous side. Hence, evidence exists for the migration of chondroprogenitor cells from the joint cavity to the superficial cartilage, both during development and during joint repair in early osteoarthritis.

were found in a location juxtaposed to the joint cavity, whereas a second putative CD271$^+$CD73$^+$ population of MSCs resided in the subsynovium[70]. Furthermore, CD271$^+$ pericytes did not seem to represent a pool of MSCs *in vivo*[70], thus contradicting the hypothesis that all MSCs could be pericytes[71]. Interestingly, human synovial fluid-resident MSCs, which are found topographically near to the synovial lining, also have a CD271$^+$CD44$^+$ phenotype[72]. Joint adipose tissues, including subsynovial fat and the infrapatellar fat pad, are also sources of MSCs[73], as are other joint structures, including the ligaments. A 2016 study provided proof-of-concept evidence that fibrous synovium-derived MSCs can be spontaneously released and might more readily access cartilage than MSCs from subsynovial adipose tissue, because more of the former cell type were released from the synovium in a novel *in vitro* assay[74].

Synovial fluid-resident MSCs. Synovial fluid-resident MSCs were found to exist at a frequency of ~40 cells per million mononuclear cells in synovial fluid from patients with OA, compared with ~1–2 cells per million in synovial fluid from patients with rheumatoid arthritis[63]. Subsequently, the frequency of synovial fluid-resident MSCs was found to be increased in patients with early knee OA with concomitant cartilage defects compared with individuals with knee pain and an absence of such lesions[75]. Another study then directly linked the number of synovial fluid-resident MSCs to the degree of chondropathy (as determined by arthroscopy) and radiographic damage[76]. MSCs are also present in gelatinous Heberden nodes (a very early lesion in hand OA) in radiographically normal joints[77], and in the synovial fluid of healthy individuals[78]. Numbers of synovial fluid-resident MSCs increase in response to

ligament and meniscal injury[76,79,80] and increased numbers of MSCs have also been reported in superficial zone cartilage[56] and subchondral bone[49] from patients with OA. Collectively, these results suggest a natural, albeit ineffective, potential for repair. Independent studies involving the intra-articular administration of culture-expanded MSCs into synovial fluid have so far shown the successful engraftment of these cells into injured ligaments[79], damaged meniscus[81] and cartilage defects[82,83], providing proof-of-principle that joint-resident MSCs might contribute to the repair of accessible joint structures. Gene profiling of cultured synovial fluid-derived MSCs and comparisons with synovium-resident and bone marrow-resident MSCs suggest that synovial fluid-resident MSCs probably originate in the synovium[76,77]. Given the size of human synovial joints, the relocation of synovium-resident MSCs to the fluid compartment provides a mechanism for 'long-range' movement to access injured cartilage and other tissues.

The identification of MSC populations in multiple tissues within the joint, including the synovium, joint adipose tissue, synovial fluid and superficial zone cartilage, which either occupy the cartilage or are in close proximity to it, challenges the idea that bone marrow-resident MSCs are absolutely necessary for cartilage repair, especially for early lesions. Numerous studies have failed to observe circulatory MSCs in health[84] or in trauma[85], and only limited engraftment of bone marrow-derived MSCs has been observed in joint surface injuries in mouse models[86], providing little support for the idea of a biological role for bone marrow-resident MSCs (mediated by systemic circulation) in cartilage repair (FIG. 3b). The existence of multiple juxta-cartilage sources of MSCs offers a different paradigm to the classic multipotent bone marrow-resident MSC model, which could be further exploited for therapy development.

An MSC model for OA cartilage repair

In contrast to the bone marrow-resident MSC model, supportive roles in osteogenesis or HSC function would not be a requirement for superficial zone MSC-mediated cartilage repair. Indeed, cultured synovial-derived MSCs have consistently good *in vitro* chondrogenic capacity compared with bone marrow-derived MSCs[67]. Moreover, cultured joint cavity-derived MSCs have superior chondrogenetic properties compared with MSCs derived from cultured subcutaneous fat[68]. In 2016, native MSCs obtained from the stromal vascular fraction of subcutaneous fat were used to treat cartilage defects as part of a microfracture procedure in patients with OA, with encouraging short-term results[87]. Whether native MSCs from a non-joint environment will be comparable to native joint-cavity derived MSCs in a therapeutic setting is an open question that needs further investigation.

These collective observations about appositional cartilage growth and the close links between cartilage and synovium raise the question of whether MSCs originating from synovium, joint adipose tissue or synovial fluid could contribute to cartilage repair in humans (FIG. 3b). Studies in a canine model of chondrogenic OA showed that MSCs injected into the synovial fluid are capable of adhering to injured cartilage[82]. Previous studies in goats also indicated that MSCs injected into the synovial cavity following meniscus excision contributed to neo-meniscus formation and integrated into adjacent synovium[81]. Culture-expanded synovium-derived MSCs can also contribute to the repair of full-thickness cartilage defects, although this observation occurred following surgical implantation of MSCs, rather than by a spontaneous repair process[88].

Although many stem cell niches, such as the skin and the gut, have respective epithelial progenitor cells located deep within the tissue, the same does not hold true for cartilage (FIG. 4). Given that cartilage damage can begin superficially, MSCs in the joint cavity are well placed to participate in early repair mechanisms. This model is reminiscent of tooth biology, in which crystals secreted into the mouth cavity from the salivary glands repair the tooth from the outside[89] (FIG. 4).

Three different strands of evidence from animal models strongly support the pre-eminence of synovium-derived MSCs in cartilage repair. First, an 'influx model' was proposed that might be important to joint development, whereby waves of migratory GDF5$^+$ cells replenish developing cartilage, rather than there just being a single layer of such cells in the early interzone[90]; however, the precise periarticular origin of such cells was not defined. Second, a 2017 study showed that *Gdf5* lineage cells in adult mammalian synovium had MSC-like proliferative features *in vitro* and contained chondroprogenitor cells that participated in post-injury cartilage repair *in vivo*[91]. In this model, cartilage repair still took place after the function of cells expressing *Gdf5* was knocked down by the conditional repression of the transcriptional regulator Yap, which might reflect involvement of stem cells from other niches, including bone marrow, owing to the thin nature of murine cartilage, although this possibility was not addressed in this model[91]. Third, another 2017 study in mice showed that the filling of cartilage defects following injury was most notably caused by synovial *Prg4*$^+$ cells, with the authors describing such cells as pioneers for cartilage repair[92].

Ageing and inflammation in MSCs

Analagous to adult stem cells, bone marrow-resident MSCs decline functionally with advancing age[43]. Likewise, several studies have shown that aged human chondrocytes are incapable of generating highly proliferative chondrogenic cells or MSCs *in vitro*, in contrast to the adjacent fat pad, which did contain functional MSCs[93]. The results of other studies suggest that only cells derived from tissues with intrinsic chondrogenic capabilities can make cartilage *in vitro*[94], an idea that cautions against the concept that cells from external sources will be capable of generating robust cartilage *in vivo*. Nevertheless, stromal cells obtained from abdominal lipoaspirates might be capable of contributing to bone repair tissue in humans[95], raising the possibility that non-cartilaginous stromal cells could differentiate along a chondrogenic lineage *in vivo* under the correct environmental cues. It remains to be seen whether stromal

cells that are not native to the joint could ultimately find a role in one-stage cartilage repair procedures, especially given the abundance of, and underappreciated importance of, joint-resident MSCs.

The potentially negative effects of joint inflammation in OA could detrimentally hinder repair and have been reviewed elsewhere[96]. The evidence that chronic synovial inflammation is ultimately detrimental to joint homeostasis is fairly compelling; however, for tissue injuries elsewhere (including bone fractures), an initial inflammatory reaction is a key part of the repair process. Evidence exists that cultured MSCs derived from an inflammatory joint environment have reduced chondrogenetic potential *in vitro*[97] and an enhanced

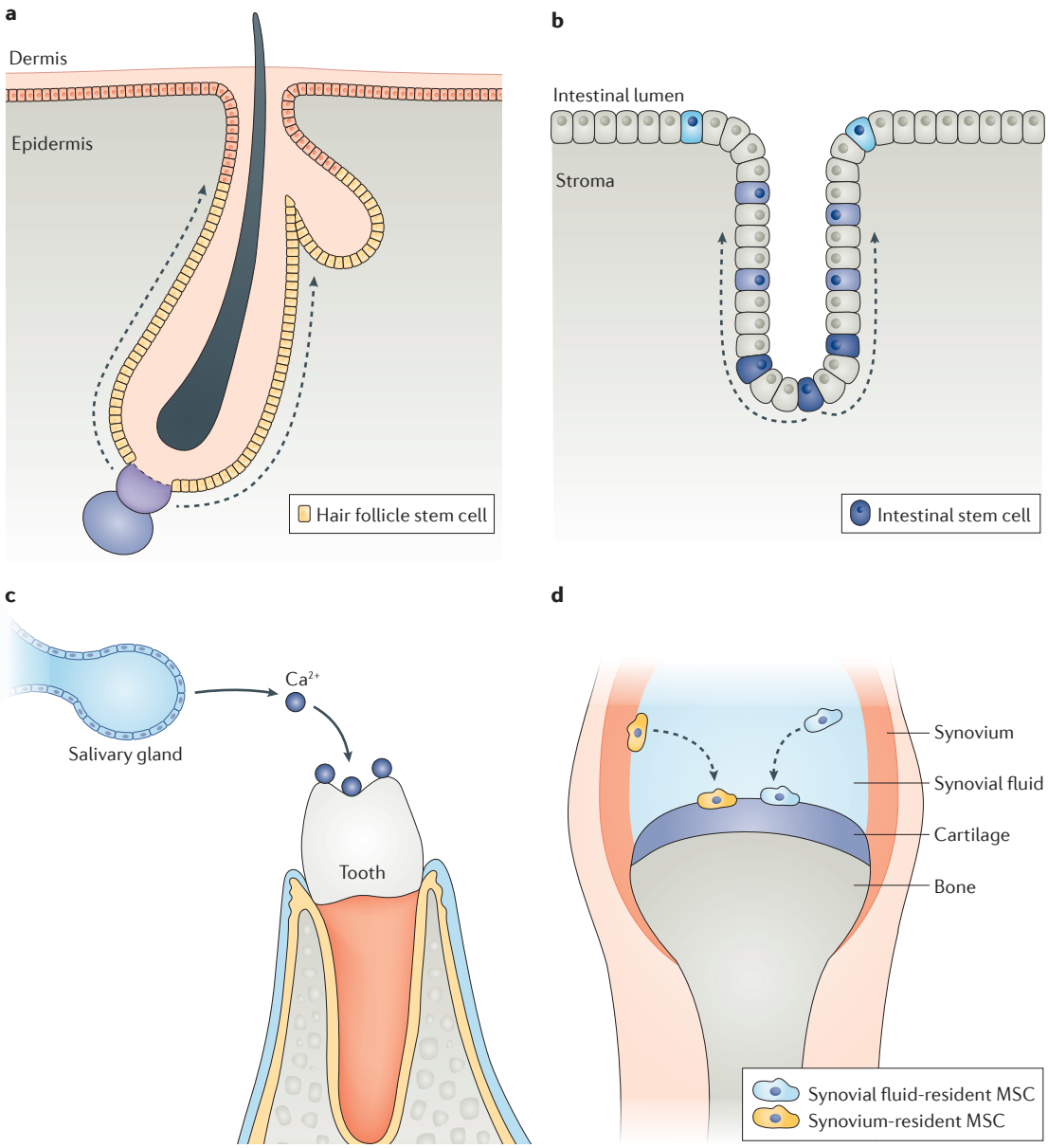

Figure 4 | **Tissue repair mechanisms differ depending on location and tissue microenvironment.** For the skin (part **a**) and gut (part **b**), which are exposed to hostile environments, there is a well-established paradigm for tissue to be repaired by basally located stem cells in the hair follicle and in the intestinal crypts, respectively. These stem cells are protected from the hostile environment. For the repair of (relatively) acellular musculoskeletal tissues, there is a completely different concept. For example, the secreted contents of saliva are involved in superficial tooth repair[89] (part **c**). The discovery that the joint cavity has several mesenchymal stem cell (MSC) niches, including the synovium and synovial fluid, and the demonstration that synovial fluid-resident MSCs can adhere to injured cartilage suggest that a model similar to that of superficial tooth repair could potentially be pivotal in cartilage repair (part **d**). In the case of cartilage repair, matrix-depositing MSCs first adhere to the most superficial tissue, which has the greatest propensity for injury. Osteotomies and joint distraction procedures enable such superficial cell-related repair mechanisms to manifest once abnormal joint mechanics have been corrected.

pro-inflammatory phenotype[98], although it is unclear whether MSC functionality reverts to 'normal' following the resolution of inflammation. With respect to synovial fluid-resident MSCs, transcriptional dysregulation that correlated with levels of monocyte chemotactic protein 1 resulted in blockade of *in vitro* chondrogenesis[78]. In murine models, inflammation is associated with an increased number of MSCs in adjacent joint fat pads[99], which is consistent with the observation in humans that the proliferation of mesenchymal lineage cells is not suppressed by inflammation[97]. In humans, synovial inflammation might be associated with the degradation of high-molecular-weight hyaluronan, which removes the anti-adhesive coating from synovial fluid-resident MSCs, thereby enabling them to adhere to cartilage[83]. These emerging insights support the idea that a degree of 'controlled inflammation' in the osteoarthritic joint microenvironment might not be detrimental to the repair process, and that the process of inflammation might provide a window of opportunity for initial MSC interactions with injured tissue (FIG. 5a).

Implications for therapy

For bone repair strategies in the clinic, there is a strong interest in the use of native, unmanipulated bone marrow-derived MSCs for one-stage fracture repair procedures, rather than using *ex vivo* culture-expansion protocols[45]. Indeed, one-stage joint repair procedures

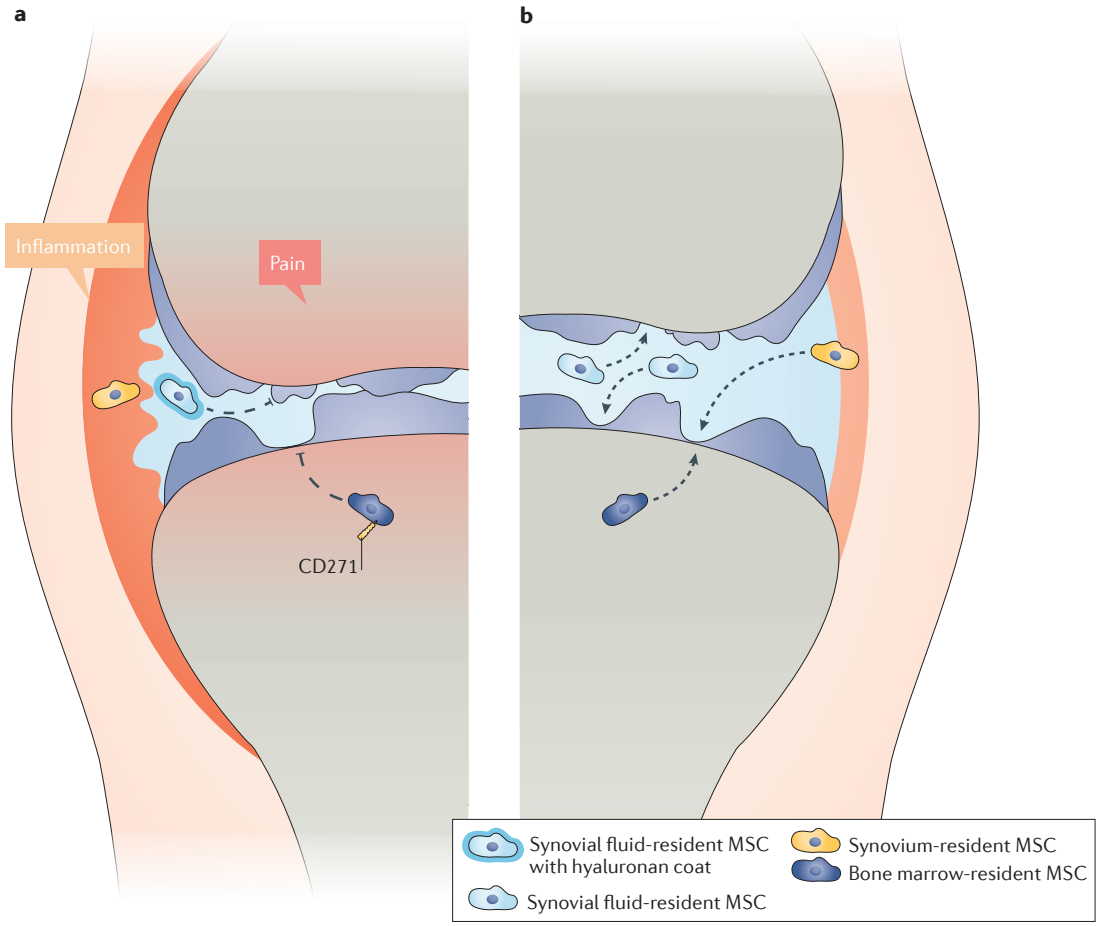

Figure 5 | **Endogenous factors influencing mesenchymal stem cells in adult cartilage repair.** Given the abundance of native or endogenous non-culture expanded mesenchymal stem cells (MSCs) that reside in the joint milieu, it will be important to elucidate the factors that govern MSC function *in vivo*. **a** | Joint inflammation, which is almost always viewed as detrimental in osteoarthritis, might be important for regulating the anti-adhesive hyaluronin coating seen on synovial fluid-resident MSCs. Limited evidence supports the idea that mitogens and growth factors introduced into the joint cavity can augment repair, but the mechanisms involved are poorly understood in humans *in vivo*. Native bone marrow-resident MSCs express CD271 (also known as low-affinity nerve growth factor receptor; LNGFR) and are especially numerous at sites of bone marrow oedema that are associated with a poor prognosis. Whether MSC-related dysfunction and neurotrophin pathways contributes to osteoarthritis awaits further studies. **b** | Joint realignment with the correction of abnormal biomechanical loading is important in cartilage repair, given that realignment osteotomies and joint distraction techniques can lead to spontaneous joint repair. Restoration of the biomechanical forces involved to a 'normal' level (for example, by realignment osteotomy) and alterations in the biochemical environment of the joint, including the loss of the hyaluranon coating from synovial fluid-resident MSCs, might therefore provide a window of opportunity in which joint-resident MSCs can repair tissues.

One-stage fracture repair
A single orthopaedic intervention to correct mechanical instability that optimizes strategies for rapid repair to prevent the need for further interventions.

that utilize knowledge of the joint microenvironment in conjunction with various factors, including joint mechanics, growth factors, biomaterials, proteases and a knowledge of how to integrate cells with adjacent cartilage and subchondral bone, might be a tenable solution for joint regeneration[100] (FIG. 5b). One-stage techniques aimed at cartilage regeneration have so far relied on bone marrow aspiration and scaffolds[101], rather than on a knowledge of *in vivo* joint-resident MSCs. In a rabbit model, the size of the holes drilled into the marrow had a large effect on the number of fibroblast colony-forming units that appeared in the microfracture-related clot[102]. Indeed, the average number of MSCs released for a 4 mm osteochondral junction breach in this model was ~300 cells[102]. Extrapolating from such animal models, we would surmise that for full-thickness lesions in human OA, bone marrow-resident MSCs might have a substantial role given that numbers of MSCs are increased in the adjacent bone[49].

The highly proliferative nature of MSCs and their chondrogenic differentiation capabilities would seem advantageous for cartilage repair compared with the limited proliferative and differentiation potential of mature chondrocytes, especially in the ageing skeleton. To repair micro-defects in superficial zone cartilage, the use of unipotent chondrocytes or joint cavity-resident MSCs would seem to be perfectly satisfactory. This idea represents a completely different paradigm for joint-repair models, which had been extrapolated from the HSC and bone marrow-resident MSC models, as only limited, unipotent chondrogenic differentiation is required for cartilage repair. The lack of specific MSC markers might attest to the fact that cartilage repair mechanisms can involve many types of cells from diverse locations in and around the joint, as set out in FIG. 3b. Another obvious implication of the multiple niches of MSCs in the joint cavity is that synovial-resident MSCs, rather than bone marrow-resident MSCs, could be used in cartilage repair, an idea that has already been tested in humans in experiments using culture-expanded synovium-derived MSCs[88].

Native CD271⁺ MSCs in OA. In patients with advanced hip OA, native CD271⁺ MSCs are fivefold more abundant in regions of MRI-determined bone marrow oedema compared with adjacent non-oedematous trabecular bone[49]. However, upon expansion in culture, MSCs derived from such oedematous regions had a reduced ability to proliferate and diminished osteogenic capacity. A 2017 microarray study comparing cells derived from bone marrow oedema and non-oedematous lesions from patients with knee OA showed that the former were associated with upregulation of several neuronal growth-related transcripts, the most highly upregulated gene being *STMN2*, which encodes a phosphoprotein that regulates microtubule function and responsiveness to NGF[103]. It remains to be determined whether this finding specifically relates to the MSC populations, which were previously shown to be more abundant in MRI-determined bone marrow oedema lesions[49]. Some studies have also suggested that native bone marrow-resident MSCs express neuronal cell adhesion molecule[104], which was originally used as a marker to define cells of neuronal lineage. Collectively, these findings are noteworthy since NGF blockade has been linked to the development of rapidly progressive OA, a fact that led to a temporary hold being put on all clinical trials of anti-NGF therapy[105–107]. Although rapidly progressive OA has been attributed to a loss of protective pain reflexes and overuse of the joints, the abundance of native bone marrow-resident MSCs in sites of bone marrow oedema and the expression of proteins originally defined in neurogenesis[103] raises the possibility of an elaborate interconnection between pain and tissue regenerative processes. Although not yet established for native bone marrow-resident MSCs, there is comparatively old literature showing how other cells derived from CD271⁺ progenitor cells, such as Schwann cells, contribute directly to tissue repair in an NGF–CD271-dependent fashion[108]. Furthermore, combined use of NSAIDs with anti-NGF therapy seems to increase the risk of rapidly progressive OA[107]. NSAIDs exert an inhibitory effect on MSC differentiation[109], providing support for the notion of an interconnection between anabolic prostacyclins and the NGF pathway in native bone marrow-resident MSC function in OA. Given the abundance of MSCs in hip OA lesions[49] and the known function of NGF in inducing the migration of CD271⁺ cells[110,111], the possibility that pain and tissue regenerative processes converge on native bone marrow-resident MSCs and on the neurotrophin pathways could be considered a hitherto unappreciated mechanism contributing to the role of anti-NGF therapy in the induction of rapidly progressive OA (FIG. 5a).

Conclusions

The perceived challenges to repairing cartilage and adjacent bone, including cell sources, types of scaffolds, lateral integration and bone anchorage[112], are potentially rendered obsolete in many scenarios by the realization that spontaneous MSC-mediated repair can happen *in vivo*, and that native MSCs are relatively abundant in the joint cavity. Additionally, scaffold technologies could be augmented by harnessing knowledge of these abundant sources of native MSCs. The remarkable structural repair demonstrated by total joint distraction procedures and osteotomies, as well as the topographic positioning of MSCs at sites of injury, highlight how intrinsic joint repair might be harnessed. Removing the mechanical load and the destructive forces acting on the damaged cartilage could provide a window of opportunity for joint-resident stem cells to re-establish joint homeostasis. The emergent understanding of native MSCs in the osteoarthritic joint microenvironment and of the ways to coax them to sites of injury (by biophysical or pharmaceutical strategies) has the potential to radically improve cartilage repair strategies. However, abnormal joint biomechanical stress is likely to make the joint environment hostile, so careful consideration of the biomechanics, especially early in the disease course, will be vital to enable native MSC repair strategies to function optimally.

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Author biographies

Dennis McGonagle is an academic rheumatologist who has researched native *in vivo* mesenchymal stem cells (MSCs) in health and disease for nearly 20 years, initially undertaking a PhD at the University of Leeds on this topic. As a founding member of the MSC Group at the Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, UK, he participated in the discovery of synovial fluid-resident MSCs and described their numerical aberrations in inflammatory and degenerative arthritis. The goal of the MSC Group is to harness knowledge of *in vivo* biology to modulate stem cell function towards joint regenerative strategies for rheumatology and orthopaedics.

Thomas G. Baboolal graduated with a PhD in biochemistry and biophysics from the University of Newcastle, UK in 2005 and is currently a research fellow in the Mesenchymal Stem Cell (MSC) Group at the Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, UK. Since 2010, his research has concentrated on the role of MSCs in cartilage, bone and soft tissue repair, in particular on how understanding MSC biology can enable the augmentation of tissue repair using devices, biologic therapeutics or other drugs. His research spans basic science and clinical translation, including medical device trials.

Elena Jones graduated with a PhD in experimental oncology from the Cancer Research Centre of the Russian Academy of Medical Sciences, Moscow, Russia. She obtained a Royal Society Fellowship in 1993 to study stem cell abnormalities in human leukaemias. Her postdoctoral work at the University of Leeds, UK was dedicated to gene therapy manipulation of bone marrow stem cells, including haematopoietic stem cells and mesenchymal stem cells (MSCs). In 2002, she described the $CD271^+$ phenotype of bone marrow-resident MSCs and in 2004 she discovered MSCs in the synovial fluid. She continues to explore human MSC biology and physiological responses in the context of acute and chronic injury of bone and cartilage.

Competing interests statement

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Native joint-resident mesenchymal stem cells for cartilage repair in osteoarthritis

Dennis McGonagle, Thomas G. Baboolal and Elena Jones

In the past few years, excitement has grown over the potential use of mesenchymal stem cells (MSCs) for cartilage repair, although the rarity of these cells has hampered progress. In this Review, the authors examine the potential of joint-resident MSCs as a new avenue for repair in osteoarthritis.

Enthesitis: from pathophysiology to treatment

Georg Schett[1], Rik J. Lories[2], Maria-Antonietta D'Agostino[3], Dirk Elewaut[4], Bruce Kirkham[5], Enrique R. Soriano[6] and Dennis McGonagle[7]

Abstract | Entheses are the insertion sites of tendons and ligaments to the bone surface and are essential structures for locomotion. Inflammation of the entheses (enthesitis) is a key feature of psoriatic arthritis and spondyloarthritis. To date, our conceptual understanding of enthesitis remains limited. This Review provides an insight into the pathophysiology of enthesitis, addressing the role of biomechanics, prostaglandin E2-mediated vasodilation and the activation of innate immune cells in the initiation phase of enthesitis, as well as the role of entheseal IL-23-responsive cells that augment inflammation by producing pro-inflammatory mediators such as IL-17A, IL-22 and TNF. In addition, the molecular steps that translate inflammation into resident tissue responses, resulting in new bone formation, are discussed. The second part of the article summarizes the clinical features of enthesitis, and the role of clinical and imaging instruments in detecting enthesitis are discussed together with their challenges and limitations. Finally, the Review summarizes the current treatment possibilities for enthesitis based on the aforementioned pathophysiological concepts, focusing on the role of cytokine-blocking agents.

Inflammation of the tendon insertion sites into bone (enthesitis) is an important and frequent manifestation of inflammatory musculoskeletal disease. Traditionally, enthesitis has received only limited scientific and clinical attention. However, this unfavorable situation has substantially changed in the past few years. In particular, the ongoing progress in the molecular characterization of diseases such as psoriatic arthritis (PsA) and spondyloarthritis (SpA), which share enthesitis as a hallmark clinical feature, have fostered our knowledge and understanding of enthesitis. In this Review, we reflect on these developments and address the latest insights concerning the pathophysiology, clinical manifestations and treatment of enthesitis. We introduce a mechanistic disease concept of enthesitis that highlights the specific pathways involved in mounting entheseal inflammation and in triggering local tissue responses. Based on this concept, we then discuss the clinical presentations as well as the diagnostic and therapeutic possibilities in enthesitis.

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Definition and function of entheses

The term enthesis derives from the ancient Greek word for insertion. In medical terminology, enthesis describes the insertion of tendons and ligaments into the bone surface[1]. Entheses are essential structures for the transduction of mechanical forces from muscles to bones and hence are the basis for locomotion. Whereas joints enable the mobility of the skeletal system by constituting natural 'breaks' between the bones, entheses transduce mechanical forces to the skeletal system (in the case of tendons) and provide stability (in the case of ligaments). Entheses are usually localized outside the joints, either inserting into the periarticular bone (as with the flexor tendon insertions of the phalanges or the biceps tendon insertion) or distant from any synovial joint (such as the Achilles tendon or annulus fibrosus insertions at the vertebral bodies). Exceptions to this rule are the entheseal region inside the knee joint, where the intra-articular cruciate ligaments of the knee insert, and dominant entheseal compartments within some distinct joints such as the sacroiliac, sternoclavicular and acromioclavicular joints. In addition, the term 'functional entheses' has been coined to describe regions where the tendon is wrapped around bony protrusions (for example, the peroneus tendons)[2]. Overall, more than one hundred entheses can be found in the human body, linking 'soft' connective tissue with the 'hard' tissue of the skeleton.

Knowledge of the anatomical features of entheses is important for understanding the process of inflammation of the entheses, which is fundamentally different from synovitis. Whereas enthesitis usually occurs outside the joint, synovitis describes an intra-articular process characterized by inflammation of the synovial

Key points

- Entheses are predominantly extra-articularly localized structures that represent a key target of musculoskeletal inflammation in diseases such as psoriatic arthritis (PsA) and spondyloarthritis (SpA)
- Entheses contain a specific immune microenvironment, which is activated by a combination of factors that include mechanical stress, genetic susceptibility and microbial-triggered immune activation
- Enthesitis arises from robust activation of prostaglandin E2 and the IL-23–IL-17 axis, leading to the influx of innate immune cells and homing of inflammation into the entheses, which is followed by mesenchymal tissue responses and new bone formation
- Clinical and imaging instruments have been developed that enable the reliable detection and monitoring of enthesitis in patients with PsA and SpA
- Inhibition of the key effector cytokines of enthesitis — IL-17, IL-23 and TNF — has shown to be effective in supporting the resolution of enthesitis in PsA and SpA

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membrane (FIG. 1). Notably, enthesitis and synovitis can occur separately or concomitantly in diseases such as PsA and SpA.

Structure of entheses

Entheses are distinct anatomical structures that are sometimes also termed entheseal complexes[3]. These structures have to enable not only the stable anchoring of the tendon or ligament into the bone surface, but also the smooth transduction of mechanical forces. These functions are made possible by the unique tissue properties of entheses, which arise from a gradual transition from tendon or ligament tissue to bone tissue as well as from specific features of cortical bone that allow a robust connection. Key insights into the microanatomy of these structures have been gained in the past 10 years, such as the appreciation that the distinct orientation of fibres in the inserting tendon allows for the physiological transduction of force[4] and the definition of a critical 500 µm-thick transition zone between the tendon and bone, where the alignment of these fibres is lost and collagen content decreases[5,6]. This region is unique in that it contains intermingled fibre-rich areas with interspersed fibroblasts and areas of chondrocytes with cartilagenous matrix[5,6]. Accordingly, collagen type I content is low in these areas, whereas collagen type II and hyalectan-type proteoglycans, such as aggrecan and versican, are increased[6]. The tissue in this transition zone between the tendon and the bone is also known as fibrocartilage[7,8]. This mixture of fibrous with cartilaginous tissue elements provides at the same time both stiffness and elasticity, which are required to fulfil the high mechanical demands on entheses. In close vicinity to the bone surface, fibrocartilage is then mineralized before transitioning into bone. Furthermore, bone at entheseal sites is thin and porous with blood vessels emerging from the neighbouring bone marrow, enabling the supply of the entheses with nutrients[2].

Pathophysiology of enthesitis

Enthesitis can result from repeated mechanical overloading, such as that which occurs during sporting activities, in otherwise healthy individuals. 'Tennis elbow' or 'golfer's elbow' is a typical example of an isolated enthesitis resulting from mechanical overload. In such cases, enthesitis usually affects only one enthesis, also involves the body of the tendon and usually resolves spontaneously. However, enthesitis is also a pathognomonic feature of PsA and SpA, where it occurs frequently, often affects more than one enthesis and shows a remarkable degree of chronicity[8]. The reason why patients with PsA or SpA are susceptible to the development of enthesitis is not fully clear. There is no conclusive evidence that enthesitis that occurs in conjunction with PsA and SpA involves a fundamentally different process than enthesitis resulting from mechanical overloading. It can be hypothesized, however, that the threshold for triggering entheseal inflammation is substantially lower in patients with PsA and SpA, which allows the development of enthesitis with little or no mechanical force — resembling an excessive reaction to low-level mechanical strain. Similar processes are known to occur in psoriatic skin disease. The well-known Koebner phenomenon describes an exaggerated and persisting inflammatory reaction of the skin to mechanical irritation in patients with psoriasis. Translated to the musculoskeletal system, enthesitis in patients with PsA and SpA might represent a pathologically exaggerated bodily response to stress. The cause for the apparently low threshold for the development of enthesitis in patients with PsA and SpA, however, remains speculative. Potential explanations include genetic factors, such as MHC class I genes and polymorphisms in *IL23R* (encoding IL-23 receptor (IL-23R)), which lead to enhanced and prolonged immune activation[9,10], and the possibility of disturbed epithelial barrier function due to concomitant clinical or subclinical psoriasis (in PsA) and colitis (in SpA), which result in increased exposure to microbial stress and prolonged immune responses[11].

Induction and inflammation

Enthesitis seems to be triggered predominantly by an innate immune response; B cell activation, follicular reactions and autoantibody formation are absent. Clinical observations suggest that mechanical stress is a central

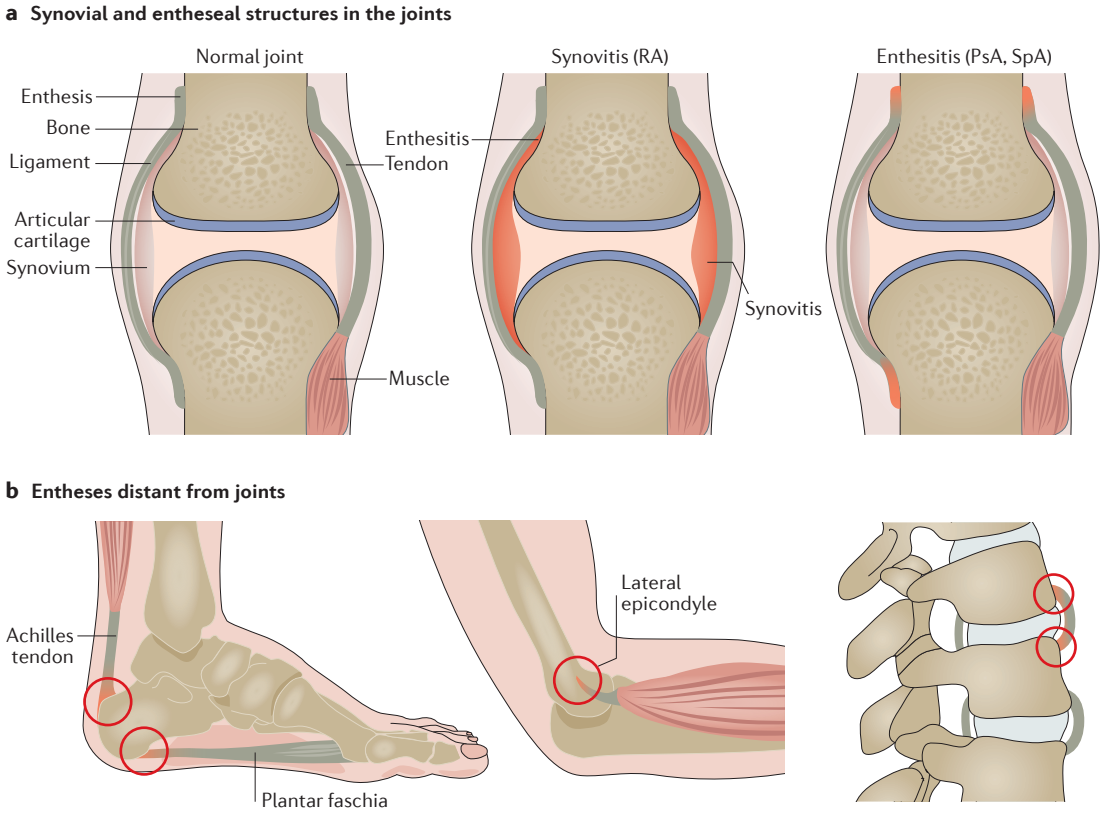

Figure 1 | **Enthesitis versus synovitis. a** | Schematic drawing of a diarthrodial joint showing the joint caspsule with the synovial membrane and tendons inserting into periosteal bone. Synovitis is characterized by inflammation of the synovial membrane. Enthesitis is defined as inflammation of the entheses, the insertion sites of tendons and ligaments to the bone surface. Enthesitis can occur with secondary synovitis. **b** | Enthesitis is usually periarticular but can also occur at sites distant from the joints (indicated by red circles), such as the Achilles tendon, the plantar fascia, the epicondyle or the anterior longitudinal ligament insertion at the vertebral edges. PsA, psoriatic arthritis; RA, rheumatoid arthritis; SpA, spondyloarthritis.

factor in the induction of enthesitis. Thus, enthesitis primarily affects the lower limbs, which are exposed to higher mechanical forces than the upper limbs[12]. In support of this concept, mechanical unloading in mice is sufficient in reducing Achilles tendon enthesitis[13]. The exact molecular process by which mechanical stress elicits enthesitis, however, remains to be determined.

An important early mediator of enthesitis is prostaglandin E2 (PGE2) (FIG. 2a). The role of PGE2 in enthesitis is supported by the remarkable responsiveness of axial and peripheral enthesitis to treatment with NSAIDs. Local PGE2 production might enable a rapid stress response to mechanical overload or other triggers in the entheses. Resident mesenchymal cells, for instance, express inducible prostaglandin G/H synthase 2 (also known as cyclooxygenase 2), which explains the site-specific production of PGE2, which is the main enzymatic product of cyclooxygenases[14]. PGE2 triggers vasodilatation, which might also widen the transcortical vessels and facilitate neutrophil recruitment from the bone marrow into the entheseal compartment. Such a process would explain the development of an inflammatory reaction in the neighbouring bone marrow (osteitis), which is observed on MRI scans of patients with PsA and SpA and is usually associated with pain (discussed further below). Furthermore, PGE2 fosters the production of IL-17 by T cells and thereby links initial inflammatory responses to activation of the IL-23–IL-17 pathway[14].

Mechanistic studies in mice have suggested that IL-23, a cytokine derived from macrophages and dendritic cells, has a key role in enthesitis. Hence, overexpression of IL-23 *in vivo* triggers enthesitis, seemingly bypassing the need for mechanical overload[15]. Notably, entheses harbour IL-23-responsive cells[16]. In mice, T cells that express IL-23R reside at entheseal sites[15,17]. Rigorous phenotyping of these cells revealed that most do not belong to the classical αβ T cell receptor-bearing group of T cells but are, in fact, γδ T cells[17]. γδ T cells are at the crossroads of innate and adaptive immunity and are instrumental in mediating host defense. γδ T cells are known to represent a major cellular source of IL-17 and TNF[18]. Whether other IL-23R-expressing cells populate entheseal sites remains to be confirmed. Some evidence suggests that innate lymphoid cells (ILCs) are interesting candidates in this respect[19]. These cells do not express a T cell receptor but share cytokine activation pathways with specific T cell lineages. Type 3 ILCs, for instance, express IL-23R, produce IL-17A and can be found in normal human entheses[20]. A functional role of these cells in enthesitis, however, remains to be determined.

The production of IL-17 seems to be a crucial step in augmenting the inflammatory response in the enthe-ses. IL-17 fosters neutrophil migration and activation, a process that is also observed in psoriatic skin disease and links IL-23–IL-17 activation with the effector phase of inflammation[21] (FIG. 2b). IL-17 acts as an amplifier of enthesitis and induces the production of a variety of cytokines and mediators by resident mesenchymal cells, which can trigger neutrophil migration and activa-tion[15,22–25]. Among these products are pro-inflammatory cytokines such as granulocyte-macrophage colony stim-ulating factor, IL-6 and IL-8, the last of which is a major chemoattractant for neutrophils. Neutrophils seem to be important effector cells in entheseal inflammation. In the enthesis, neutrophils further augment the inflam-matory response by releasing proteases and reactive oxygen species, which aggravate pain responses during enthesitis. Very few histopathology studies have been done in human enthesitis. These studies suggest that also macrophages infiltrate into the entheseal tissue[26]. The activation state of neutrophils and macrophages is critical in determining the development of enthesi-tis. For instance, uncontrolled activation of signal transducer and activator of transcription 1 (STAT1) in myeloid cells has been shown to trigger enthesitis by promoting cytokine release. In the absence of A20 pro-tein, a negative regulator of STAT1, enthesitis develops spontaneously[27].

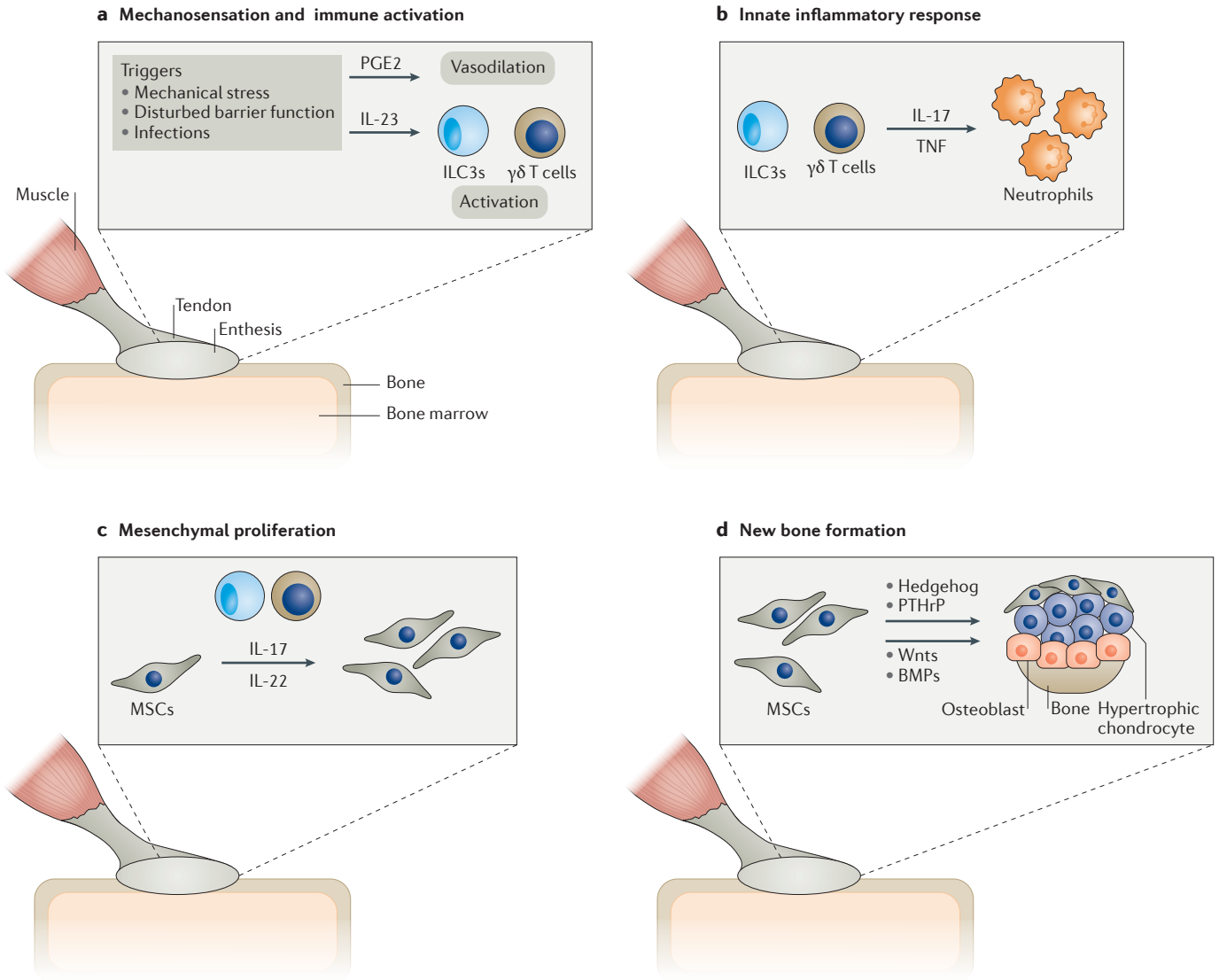

Figure 2 | **Functional model of enthesitis. a** | Enthesitis is initiated during a mechanosensation and immune activation phase involving mechanical and/or infectious stress that leads to the activation of prostaglandin E2 (PGE2) and IL-23, followed by vasodilatation and activation of resident γδ T cells and type 3 innate lymphoid cells (ILCs). **b** | The subsequent innate inflammatory response is characterized by the release of TNF and IL-17, leading to the influx of immune cells such as polymorphonuclear neutrophils (PMNs). **c** | Mesenchymal proliferation elicited by IL-17 and IL-22 is characterized by the activation and proliferation of resident mesenchymal stem cells (MSCs) from the perientheseal periosteum. **d** | New bone formation at entheseal sites (enthesophyte growth) is triggered by hedgehog signalling and parathyroid hormone related-peptide (PTHrP), which contribute to the mineralization of fibrocartilage; bone morphogenic proteins (BMPs) and Wnt proteins induce osteoblast differentiation and enable new bone apposition.

Tissue proliferation and bone formation

Entheseal inflammation is characterized by remarkable tissue responses (FIG. 2c). Central to these architectural changes is local new bone formation. Although spurious erosions can occur in the context of enthesitis, the net effect of entheseal inflammation is the gain of new bone, which is often characterized by excessive local apposition of periosteal bone at entheseal sites (enthesophytes)[28,29]. In the spine, the anterior and posterior parts of the vertebral bodies are affected, which leads to syndesmophyte formation and subsequent spinal ankylosis[30]. At peripheral sites, entheses such as the plantar fascia are affected by new bone formation (calcaneal spurs). Furthermore, entheseal sites at peripheral joints such as the hand joints give rise to new bone formation in PsA[29]. Remarkably, the first sign of musculoskeletal involvement in patients with psoriasis is enthesophyte formation in the peripheral joints, highlighting the role of enthesitis as an early feature of diseases such as PsA and SpA[28].

Mechanistically, new bone formation is speculated to represent a tissue response process that starts after the peak of entheseal inflammation has been reached. This process is probably initiated by resident mesenchymal cells, which have the potential to proliferate and differentiation into chondroblasts and osteoblasts to form cartilage and bone, respectively. In some respects entheseal new bone formation resembles fracture repair, which is characterized by a rapid and robust mesenchymal tissue response following an initial inflammatory phase[31]. The molecule(s) linking the inflammatory phase with the tissue response in enthesitis are as yet unknown but IL-17, IL-22 and PGE2 have been implicated in this process. IL-17, for instance, has shown to effectively activate mesenchymal cells[32,33]. Furthermore, although epithelial cells are the key targets of IL-22, other resident entheseal cells, such as mesenchymal cells, also respond to this cytokine and IL-22 might therefore support new bone formation[34]. Finally, PGE2 is a robust activator of osteoblast differentiation and hence might link entheseal inflammation with new bone formation[35]. By contrast, TNF seems to be primarily anti-anabolic through its induction of Dickkopf-related protein 1 (DKK1) and sclerostin, both of which effectively block bone formation[36,37]. Although there is experimental evidence that the absence of TNF retards fracture repair, the clinical relevance of this observation remains unclear[38].

In contrast to the initiation process of enthesophyte growth, the factors required for chondroblast and osteoblast differentiation are well characterized (FIG. 2d). Hedgehog proteins activate a specific cell population in the enthesis (cells expressing the hedgehog-regulated transcription factor GLI1), which are different from the tendon fibroblasts[39,40]. These GLI1$^+$ cells are critical for building mineralized fibrocartilage and their activity seems to be controlled by muscle loading. Accordingly, inhibition of smoothened homologue (SMO), a key component of hedgehog signalling pathways, has been shown to block enthesophyte formation[41]. Furthermore, parathyroid hormone-related peptide is also expressed in the entheses and probably supports the recruitment and/or activity of underlying bone cell populations[42].

Osteoblast differentiation and new bone formation are facilitated by bone morphogenic proteins (BMPs) and Wnt proteins. Increased BMP and Wnt expression has been associated with excessive new bone formation and the generation of bone spurs. For instance, BMP2 is expressed by mesenchymal cells of the entheses and BMP6 and BMP7 are expressed during the later stages of chondrocyte differentiation[43]. Similarly, both murine and human entheses show activation of SMAD1–SMAD5 during inflammation, indicative of active BMP signalling[44]. Inhibition of the BMP signalling pathway by noggin retards new bone formation in male DBA1 mice, a model of enthesitis characterized by enthesophytes[44]. Thus, BMPs seem to essentially promote the proliferation of mesenchymal precursors, which are required to form hypertrophic chondrocytes. These cells build the scaffold for the later apposition of new bone by osteoblasts, which form the enthesophyte. Wnt proteins and their inhibitors, DKK1 and sclerostin, are key effector molecules for osteoblast activity and enable new bone apposition at entheseal sites[36,37]. The balance between Wnt proteins and their inhibitors is also crucial for the amount of new bone formed at entheseal sites. For instance, blockade of the Wnt inhibitor DKK1 is associated with more pronounced differentiation of mesenchymal stem cells into hypertrophic chondrocytes, resulting in bony spur formation at peripheral joints as well as ankylosis of the sacroiliac joints[36,45].

Detection and assessment of enthesitis

Prevalence and clinical presentation

In a 2016 publication, Polachek and colleagues provided a detailed analysis of the prevalence and clinical presentation of enthesitis in >800 patients with PsA[46]. Enthesitis was defined as pain at specific tendon insertion sites (such as the Achilles tendon or the plantar fascia) using established clinical instruments, which are described below. The prevalence of enthesitis in patients with PsA in this study was 35%, with the Achilles tendons, plantar fasciae and lateral epicondyles being the most commonly involved sites. Additional studies have suggested that enthesitis can be among the first symptoms of PsA and SpA[47,48]. High body mass, more active joint disease and young age are factors associated with the appearance of enthesitis. The association of young age with enthesitis might indirectly implicate mechanical load as a trigger of entheseal inflammation, as physical activity is usually higher in younger individuals than older individuals[46].

Whereas the prevalence of enthesitis (defined as pain at defined sites) seems to be between 30% and 50% in patients with PsA, the overall burden of enthesitis might in fact be higher for two reasons. Firstly, entheseal sites are more abundant than those assessed in standard clinical examinations. Because entheses are also found in direct conjunction with the joints, arthralgia in PsA and SpA can sometimes result from enthesitis rather than from synovitis, hence the attribution of joint pain to synovitis can lead to an underestimation of the prevalence of enthesitis. This concept is supported by MRI studies in patients with SpA, which showed enthesitis

underlying the clinical manifestation of 'arthritis' (REF. 49). Secondly, the application of imaging rather than palpation of entheses yields a substantially higher prevalence of enthesitis, with ~70% of patients with PsA affected by enthesitis[50,51]. Hence, the overall clinical burden of enthesitis in patients with PsA and SpA is high and possibly still underestimated[52].

Clinical assessment

Traditionally, the presence of enthesitis is ascertained by clinical examination. Clinically, the only way to assess the presence of enthesitis is to assess tenderness at the entheseal site. However, it is not clear whether tenderness always denotes inflammation, nor it is clear whether the absence of tenderness rules out enthesitis. In contrast to synovitis, where swelling in addition to tenderness is an important discriminator between inflammation and pain, swelling is absent in enthesitis with the exception of bony enlargement resulting from enthesophyte formation or occasional entheseal enlargement (TABLE 1). Hence, the question of whether tenderness at entheseal sites is related to hyperalgesia alone or indeed is also related to an inflammatory process is difficult to answer by clinical examination alone.

Despite these challenges, reliable clinical instruments have been developed to assess enthesitis, which add up tender entheseal points[53]. The Spondyloarthritis Research Consortium of Canada (SPARCC) index covers the 16 most relevant peripheral sites affected by enthesitis such as the Achilles tendon, plantar fascia and femoral trochanter as well as entheseal sites at the knees, elbows and shoulders[54]. The Leeds enthesitis index (LEI) is also focused on the assessment of peripheral enthesitis but is confined to only six entheses (Achilles tendon, lateral distal humerus and medial distal femur on each side of the body)[55]. The third index for clinical evaluation of enthesitis is the Maastricht ankylosing spondylitis enthesitis score (MASES), which focuses primarily on the axial entheses such as those along the ribs and the iliac crest; the Achilles tendon is the only peripheral entheseal site included in this score[56]. MASES largely replaced the rarely used and hardly feasible Mander enthesitis index (MEI), which assesses a total of 66 entheses in the spine and peripheral skeleton[57]. To date, the SPARCC, LEI and MASES indices are mostly used in clinical trials to assess the efficacy of DMARDs for enthesitis, whereas their use in clinical practice is limited. This situation might seem surprising since these easy-to-use instruments are the only clinical tools available for assessing enthesitis and because joint counts have been used successfully for many years in the clinical assessment of synovitis.

Imaging of enthesitis

The acknowledged limitations in the clinical assessment of enthesitis have prompted clinicians to search for better instruments for its detection and monitoring. Although pain is an important, if not the most important, clinical sign of enthesitis, it is not specific and hence does not prove the presence of enthesitis. Therefore, different imaging modalities have been applied to better detect enthesitis and to distinguish it from mere pain

Table 1 | **Comparison of the features of enthesitis and synovitis**

Feature	Enthesitis (PsA and SpA)	Synovitis (RA)
Anatomical localization	Extra-articular	Intra-articular
Tissue composition	Fibrocartilage	Synovial membrane
Mechanical trigger	+++	+
Aetiopathogenesis	Danger response	Autoimmunity
Resident immune cells	γδ T cells, type 3 innate lymphoid cells	Tissue-resident macrophages
Resident non-immune cells	Periosteal and fibrocartilage MSCs	Fibroblast-like synoviocytes
Type of immune activation	Innate (mostly polymorphonuclear neutrophils)	Mixed
Genetic associations	MHC class I genes, *IL23R*	MHC class II genes
Clinical symptoms	Pain	Pain, swelling
Pre-clinical phase	Subclinical enthesitis	Autoantibodies, tenosynovitis
Bone marrow involvement	+++	+
New bone formation	+++	–
PGE2 dependence	+++	+
Clinical effect of methotrexate	–	++
IL-17–IL-23 dependence	+++	+
IL-6 dependence	–	+++
TNF dependence	+++	+++
Associated organs	Gut, skin	Lungs

–, absent; +, minor; ++, moderate; +++, strong; MSC, mesenchymal stem cell; PGE2, prostaglandin E2; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SpA, spondyloarthritis-,

or the involvement of adjacent structures[58]. Assessment of enthesitis by imaging reflects the pathophysiological processes underlying enthesitis, demonstrating either the inflammatory phase or the tissue response phase of the disease.

Imaging the inflammatory phase of enthesitis. The inflammatory phase of enthesitis can bedetected by visualizing the hyperaemia and vasodilation that precedes and facilitates both immune cell migration and the deposition of an inflammatory infiltrate. A 1998 study by McGonagle *et al.* marked the first use of MRI for demonstrating the link between enthesitis and arthritis, confirming the hypothesis that enthesitis is the landmark lesion of SpA[49]. MRI is particularly useful in detecting perientheseal osteitis, which appears as soft tissue 'oedema' on short tau inversion recovery (STIR) images and fat-suppressed contrast-enhanced T1-weighted images in the bone marrow adjacent to entheses (see also the schematic drawing in FIG. 3). Detection of perientheseal osteitis by use of MRI is also of importance in the assessment of axial disease associated with PsA and particularly in SpA. MRI has revealed that osteitis is a hallmark of inflammation in axial fibrocartilagenous joints such as the sacroiliac and sternoclavicular joints as well as in the vertebral bodies in patients with axial SpA or ankylosing spondylitis[59]. Furthermore, MRI studies have also shown that extensive osteitis sometimes accompanies peripheral enthesitis, such as enthesitis adjacent to the plantar fascia, in patients with peripheral SpA[60]; similar, although less extensive, lesions have also been observed in mechanical enthesitis[60]. A study using whole-body MRI revealed the coexistence of perientheseal osteitis at axial and peripheral sites in patients with PsA and SpA[61]. Overall, MRI studies support the concept that the entheses and the perientheseal bone marrow form a functional unit. The porous cortical bone, which is characterized by multiple transcortical vessels, enables intensive communication between the bone marrow and the enthesis. In fact, the bone marrow might serve as a 'recruitment pool' for immune cells such as neutrophils entering the entheses through transcortical vessels. Vasodilatation in the perientheseal bone marrow, which appears as a water-rich signal on MRI and is probably triggered by PGE2, might facilitate this process (FIG. 3). Such a concept is strongly supported by ultrasonography studies demonstrating enhanced blood flow based on the vascularization of the enthesis–bone junction[62].

Assessment of vascularization by measuring the power Doppler signal can, to a certain extent, differentiate inflammatory enthesitis from mechanical enthesitis: whereas inflammation is directly localized at the bony insertion in the inflammatory enthesitis of PsA and SpA, the changes in mechanical enthesitis are more distant from the bone and sometimes represent tendinitis rather than enthesitis[58,60]. Ultrasonography studies have shown that enthesitis is an early feature of PsA but is not found in patients with rheumatoid arthritis[63]. Subclinical enthesitis has been detected by ultrasonography in patients with psoriasis without a history of PsA[64–66] and is more common in patients with psoriasis who have clinical nail involvement[64]. The concept of the nail–entheseal

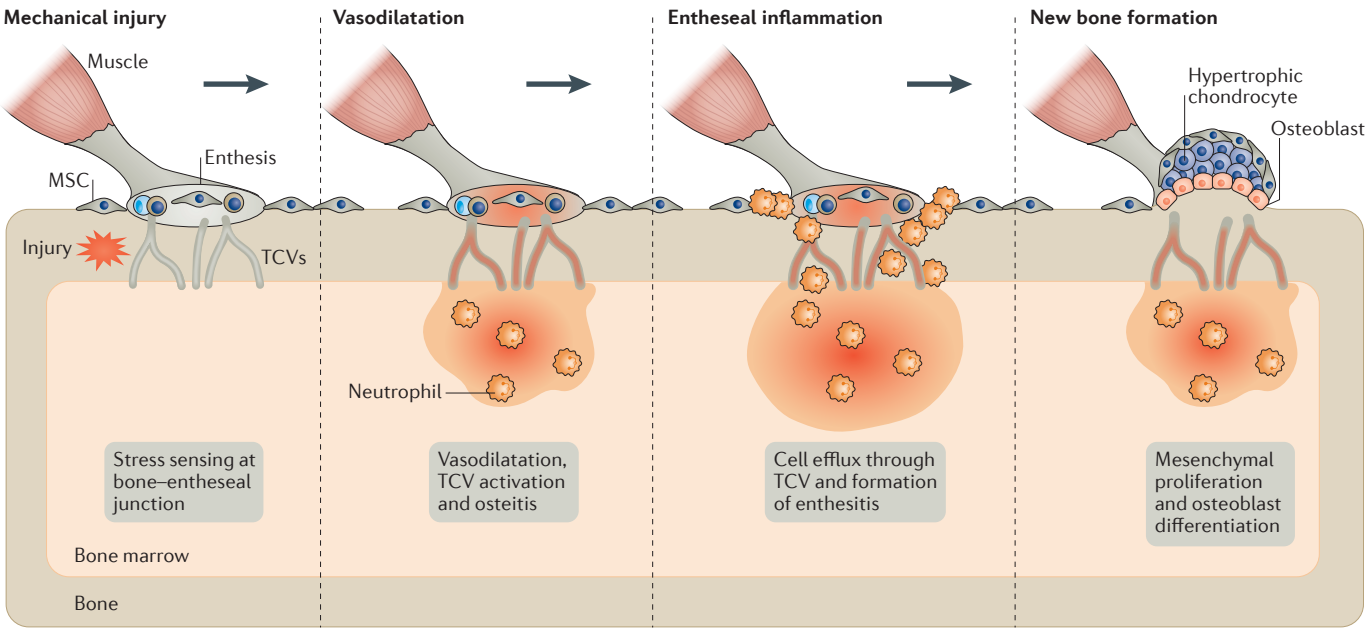

Figure 3 | **Microanatomical changes in enthesitis.** In a normal enthesis, a tendon inserts into a porous trabecularized bone, which is characterized by a high number of transcortical microvessels (TCVs) that enable communication between the bone marrow and the enthesis. The bone–entheseal junction is subject to mechanical stress (red star). Following mechanical stress at the enthesis, TCVs are activated and an inflammatory reaction (osteitis) forms in the adjacent bone marrow. TCV widening via vasodilatation facilitates the efflux of immune cells (such as neutrophils) from the perientheseal bone marrow into the enthesis.

complex is further supported by ultrasonographic signs of enthesitis in the distal interphalangeal joints of patients with psoriasis and PsA[66]. Finally, and supporting the concept of initially PGE2-induced and later cytokine-induced vasodilatation in enthesitis, several studies have shown the responsiveness of entheseal inflammation to treatment with NSAIDs and TNF inhibitors by demonstrating a decrease in the power Doppler signal[67–70].

Imaging the tissue response phase of enthesitis. The anatomical changes related to the considerable tissue responses associated with enthesitis were the focus of imaging research even before the depiction of inflammatory changes. Until 1990, conventional radiography was the only technique commonly available; therefore, the radiographic features of enthesitis have played a pivotal role in defining entheseal lesions in SpA. These features include periarticular osteopenia (most likely related to bone marrow inflammation), cortical bone irregularities and erosions at insertion sites (most likely linked to enlarged transcortical vessels), and signs of calcification and new bone formation, which are pathognomonic for enthesitis. Instruments developed to score new bone formation, such as the Stoke ankylosing spondylitis spine score (SASSS) and Rathingen score, were later used to enable the quantification of tissue responses in axial and peripheral entheseal disease, respectively[71,72]. In addition to conventional radiography, ultrasonography is particularly instrumental to investigating structural pathology in the peripheral joints, with the first study of this approach dating back to 1994 (REF. 73). Ultrasonography can depict structural lesions such as bone erosions, bony spurs and thickening of the tendon or ligament insertion[74]. Scoring instruments such as the Glasgow ultrasound enthesitis scoring system (GUESS)[75] or the Spanish enthesitis index (SEI)[76] have been developed to assess the presence and severity of enthesitis on the basis of such morphological changes, even though these findings are also commonly found in mechanical pathologies. More recently, high-resolution peripheral quantitative CT (HR-pQCT) was introduced to define structural lesions in enthesitis, in particular the quantification of new bone formation in PsA. Studies using HR-pQCT showed that entheseal new bone formation is a very early sign of musculoskeletal involvement in patients with psoriasis[28]. Furthermore, enthesophytes represent the dominant structural feature in established PsA but are virtually absent in rheumatoid arthritis. These data suggest that a large component of the structural changes seen in PsA is driven by entheseal inflammation[29]. Moreover, HR-pQCT showed that TNF inhibition arrests the progression of bone erosions but does not halt the progression of enthesiophye formation, indicating essential differences in the pathophysiology of bone erosions and enthesophytes[77]. Taken together, imaging studies using conventional radiography, ultrasonography and HR-pQCT have been essential to understanding the tissue responses associated with enthesitis that escape detection by physical examination.

Treatment of enthesitis

The most stringent proof that a certain pro-inflammatory mediator has a pathophysiological role in a disease process is that a compound inhibiting that mediator has therapeutic efficacy[78]. Notably, current knowledge on the treatment of enthesitis is limited. Remarkably, to date no study has been specifically designed to evaluate the treatment of enthesitis. DMARDs have not been studied in enthesitis and clinical trials of cytokine-blocking agents in which enthesitis instruments were applied (in those patients who actually demonstrated enthesitis) were not powered to assess enthesitis. Nonetheless, the observations on the apparent therapeutic efficacy of drugs in the treatment of enthesitis largely support the pathophysiological concepts introduced in the first part of this Review.

In clinical practice, the treatment of enthesitis aims to resolve inflammation and prevent subsequent inflammation-induced tissue responses. To date, all drugs used for treating enthesitis aim to stop entheseal inflammation and relieve symptoms. The concept that resolution of entheseal inflammation might also affect the related tissue response is, however, not well developed. Enthesitis and related osteitis often respond to NSAIDs, which are widely used in rheumatic diseases associated with enthesitis such as PsA and SpA. As mentioned, ultrasonography studies have confirmed the effects of NSAIDs on vasodilatation and inflammation at entheseal sites[68]. In fact, clinical observations suggest that enthesitis is much more sensitive to NSAIDs than is synovitis, pointing to a more dominant role of PGE2 in enthesitis than in synovitis. The effects of NSAIDs in enthesitis might rely on the inhibition of vasodilatation of transcortical and bone marrow vessels as well as the limitation of PGE2-associated pain responses. Furthermore, NSAIDs suppress tissue responses associated with enthesitis, as PGE2 is a potent inducer of osteoblasts[35]. In axial SpA, the use of NSAIDs might also retard new bone formation, although the data are not always consistent[79,80].

If enthesitis becomes chronic, NSAIDs often do not adequately control disease and additional drugs are required. Unfortunately, methotrexate does not show efficacy in inhibiting entheseal inflammation, in contrast to its well-documented action in synovitis. Similarly, other conventional DMARDs, such as leflunomide and sulfasalazine, do not seem to work in enthesitis. By contrast, the phosphodiesterase 4 inhibitor apremilast, which has been approved for the treatment of PsA, is currently the only orally available DMARD with proven efficacy in enthesitis. Apremilast inhibits the production of several cytokines involved in entheseal inflammation, such as IL-17A, IL-23 and TNF[81], and also limits the migration of neutrophils to sites of inflammation[82], thereby interfering with the key cytokines and cells involved in the onset of enthesitis. About half of the patients with PsA treated with apremilast show complete resolution of enthesitis as measured by MASES after 1 year of treatment[83]. Although these results are encouraging, further data in peripheral enthesitis is needed for apremilast as MASES focuses largely on axial rather than peripheral enthesitis, whereas other indices (such as SPARCC and LEI) better discriminate peripheral enthesitis[84].

The role of TNF inhibitors in controlling enthesitis is reflected by their well-documented efficacy in improving spinal pain in axial SpA and ankylosing spondylitis[85,86]. Spinal pain in axial SpA and ankylosing spondylitis is presumed to originate from inflammation of fibrocartilagenous entheseal joints such as the sacroiliac joints and the ligament insertion sites at the anterior and posterior bodies of the vertebrae, which are usually associated with substantial osteitis. In addition, TNF inhibitors also improve the signs and symptoms of peripheral enthesitis, such as in the heels of patients with axial SpA and peripheral entheseal involvement[87]. Accordingly, results from several clinical trials in PsA provide substantial evidence that TNF inhibitors are efficacious in controlling peripheral enthesitis: after treatment with infliximab, the number of patients with symptoms of enthesitis in the feet declined by 50% in one trial[88]; improvements in all three aforementioned enthesitis indices (SPARCC, LEI and MASES) have been reported following treatment with adalimumab[84,89]; and etanercept, golimumab and certolizumab[90–92] have proven efficacy in the treatment of peripheral enthesitis.

MRI studies have confirmed the concept that axial and peripheral entheseal inflammation are responsive to TNF inhibitors. In particular, peri-entheseal osteitis along the sacroiliac joints and in the vertebral bodies in ankylosing spondylitis resolved after TNF inhibition[93]. Furthermore, studies on peripheral enthesitis in PsA have shown that TNF inhibition improves peri-entheseal osteitis detected by MRI[94] and increased vascularization measured by power Doppler ultrasonography[62,70]. Taken together, these clinical and imaging data provide robust evidence that TNF is an effector cytokine in enthesitis.

Apart from TNF inhibition, newer data have revealed a striking responsiveness of enthesitis to inhibition of IL-23 and IL-17A. Ustekinumab, an antibody against the p40 subunit common to IL-12 and IL-23, has shown to effectively treat enthesitis in slightly more than 50% of patients with PsA after 6 months of treatment[95]. Considering that <20% of patients with PsA treated with ustekinumab achieve a high-level response (that is, ≥70% improvement in ACR response criteria) in their joint symptoms, the improvement in enthesitis is remarkable and strongly supports a central role of the IL-23–IL-17 axis in entheseal inflammation. This concept is also supported by data on IL-17A inhibition in PsA and ankylosing spondylitis: treatment with the IL-17 inhibitors secukinumab and ixekizumab results in improvements in enthesitis scores with resolution of enthesitis in ~50% of the patients treated with secukinumab and 30–40% of those treated with ixekizumab[96,97]. Overall, the remarkable clinical efficacy of inhibitors of the IL-23–IL-17A pathway in enthesitis, and the ability of these therapeutic agents to control the symptoms of axial disease in ankylosing spondylitis, supports the pathophysiologic concept of an IL-23–IL-17A pathway-dependent inflammation of entheseal structures.

Conclusions

Experimental and clinical observations reveal remarkable differences between entheseal and synovial inflammation with respect to their pathogenesis, diagnosis and treatment. Enthesitis is a distinct disease process, which can occur independently of arthritis. It relies on a multistep process consisting of initiation and augmentation of inflammation followed by local tissue responses leading to new bone formation. Mechanical stress, innate immune activation and mesenchymal tissue modelling and remodelling are hallmarks of the process of enthesitis and are guided by distinct molecules and cells. Despite the development of this mechanistic framework, which is supported by findings showing the effects of drugs targeting entheseal inflammation, there are still substantial limitations in our knowledge of how enthesitis works. Experimental modelling of enthesitis is in its infancy, especially when compared with the modelling of arthritis. Furthermore, the accessibility of human entheseal tissue is very limited, which has retarded progress in defining the key cellular and molecular players of enthesitis in human disease. Some, but not all, of these hurdles can be overcome by applying modern imaging techniques. However, further insights into the molecular and cellular players of enthesitis are also needed. We anticipate that modern immunologic research will be able to tackle some of these open questions.

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ESSAY

The brain and immune system prompt energy shortage in chronic inflammation and ageing

Rainer H. Straub

Abstract | Sequelae frequently seen in patients with chronic inflammatory diseases, such as fatigue, depressed mood, sleep alterations, loss of appetite, muscle wasting, cachectic obesity, bone loss and hypertension, can be the result of energy shortages caused by an overactive immune system. These sequelae can also be found in patients with chronic inflammatory diseases that are in remission and in ageing individuals, despite the immune system being less active in these situations. This Perspectives article proposes a new way of understanding situations of chronic inflammation (such as rheumatic diseases) and ageing based on the principles of evolutionary medicine, energy regulation and neuroendocrine–immune crosstalk. A conceptual framework is provided to enable physicians and scientists to better understand the signs and symptoms of chronic inflammatory diseases and long-term disease consequences resulting from physical and mental inactivity.

Chronic inflammatory diseases, such as rheumatoid arthritis (RA), and the process of ageing are often accompanied by shared signs and symptoms, including fatigue, depressed mood, sleep alterations, loss of appetite and context-associated anorexia, anaemia, malnutrition, muscle wasting, bone loss, insulin resistance, decreased fertility, loss of sexual interest, increased sympathetic and low parasympathetic activity, high blood pressure and hypercoagulability[1]. Given the pervasive influence of these sequelae on an individual's well-being, quality of life, functional capacity and physical and mental activity, they might be considered to constitute a long-term risk of illness and early death. The aforementioned sequelae seem to be separate manifestations; however, when viewed from a perspective of energy shortage, especially in the presence of inflammation-related and age-associated anorexia[2–5], these sequelae can be considered to be interrelated.

Inflammation is widely suspected to be central to those sequelae common to chronic inflammatory diseases and ageing. Indeed, ageing is often accompanied by an increased pro-inflammatory load[6], and chronic inflammatory diseases are known to accelerate the ageing process[7,8]. However, the low-grade inflammation seen in ageing individuals or in patients with chronic inflammatory diseases that are in remission cannot be compared to the degree of inflammation seen in patients with newly diagnosed chronic inflammatory diseases (or during disease flares)[9–14]. This comparison is true not only for direct measures of immune system activity (for instance, serum levels of IL-6), but also in terms of inflammation-induced energy expenditure[13]. Although inflammation in such newly diagnosed and active chronic inflammatory diseases can induce extra energy costs of 10–15% of an individual's total energy expenditure[1,9–12], the low-grade inflammation seen in ageing individuals or in patients with chronic inflammatory diseases that are in remission should, according to data derived from experimentally induced mild inflammation in healthy individuals[13,14], lead to maximal extra energy costs of 2–3%. If such sequelae are triggered by an energy shortage induced by energy expenditure, it follows that extra energy costs (that is, energy costs not related to inflammation) must exist during ageing or in a chronic inflammatory disease that is in remission. So what is the cause of these extra energy costs when the immune system is not solely responsible for the energy shortage? Based on the special roles of the immune system and brain in energy regulation, it is postulated that extra energy costs could be caused by increased psychomotor activity (for example, as a result of pain, stress and sleeping problems). In an update to previous models[1,15], the model proposed in this Perspectives article states that energy shortage is based on the sum of all extra energy costs (psychomotor activity plus inflammation). In ageing individuals or patients with chronic inflammatory diseases that are in remission, these extra energy costs lead to unwanted mental and physical inactivity and their associated consequences.

This Perspectives article does not aim to discuss energy regulation at the cellular level (reviewed elsewhere[16–19]) or to discuss the molecular interface between metabolism and the immune system, such as how nutrients act through pathogen-sensing and other inflammatory pathways[20]. Instead, the reader encounters an integrative approach at the level of the entire body — the bird's-eye view of an internist — to address complex symptomatology in a manner summarized by Walter B. Cannon[21]: in a healthy individual, "the internal environment is kept constant [homeostasis] so that we are freed from limitations imposed by internal and external conditions that could be disturbing." The article begins by describing the hierarchical position of the brain and immune system in energy regulation, introducing the idea of 'selfishness' and conceptualizing the interrelation between energy regulation and memory in these two systems (FIG. 1). Next, a detailed description of the concepts of energy expenditure stimulated by the immune system and energy expenditure instigated by the brain is provided, and the notions of volitional (or intentional) energy

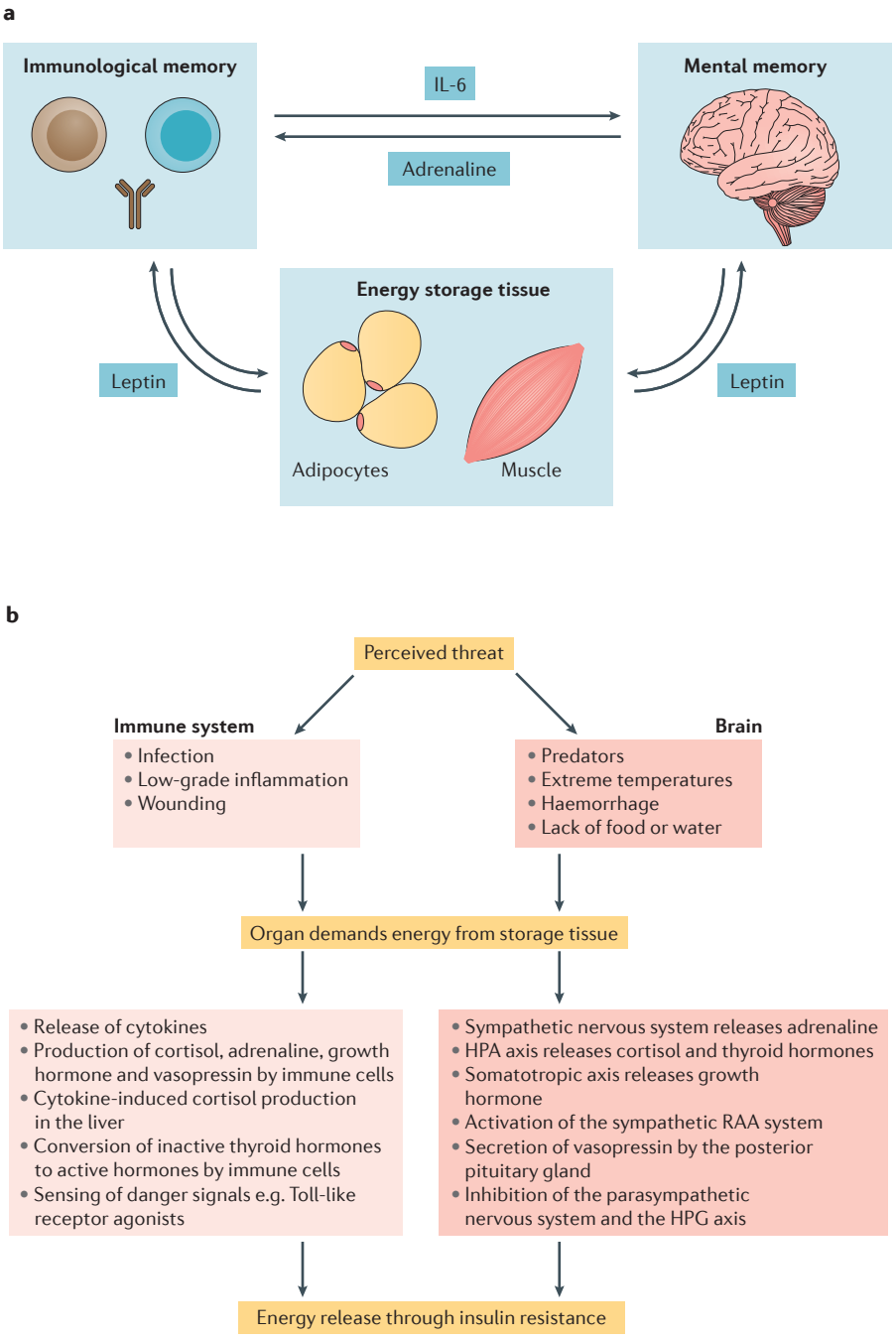

Figure 1 | **Regulation of energy storage and energy release.** Three organ systems, each of which has a form of memory, are instrumental in energy regulation, particularly in storing and releasing energy from reserves. Energy reserves provide a 'memory' of food availability, food intake, general health status and times of previous energy shortage. **a** | Energy reserves are protected by the immune system and by the brain. Signals from energy storage tissues to the immune system and the brain via leptin indicate the amount of energy stored in the body's reserves. The brain and the immune system also signal to each other by mechanisms including IL-6, adrenaline and many others. **b** | In situations in which an individual encounters short-lived energy-demanding threats, such as predators, food shortages, extreme temperatures, infectious agents, wounding and haemorrhage, either the immune system or the brain can demand energy-rich fuels by stimulating insulin resistance, lipolysis and other energy-liberating pathways. Examples of mechanisms used by the brain or the immune system to induce insulin resistance include cortisol release[92] by the hypothalamic–pituitary–adrenal (HPA) axis and also by immune cells, which can convert cortisone to cortisol locally, and vasopressin, which can stimulate lipolysis[93]. HPG axis, hypothalamic–pituitary–gonadal axis; RAA system, renin–angiotensin–aldosterone system.

expenditure (for instance, exercising at the gym) and non-volitional (or unintentional) energy expenditure[22] (for instance, an immune response against an infection) are introduced. To conclude, a model is presented that explains the appearance of long-standing problems seen in patients with chronic inflammatory diseases and during ageing as a consequence of maladaptive energy regulation.

Two 'selfish' super-systems

The main super-systems involved in energy regulation, the brain and the immune system, can be defined using concepts derived from evolutionary biology and biological anthropology[15,23,24]. Major threats to the individual, such as predators, food scarcity, thirst, extreme temperatures, infectious agents, wounding and haemorrhage are perilous aspects of life under natural conditions (TABLE 1). These threats were dominant during evolution, so can elicit immediate responses from the brain or from the immune system[15,23,24]. Since the responses to these threats demand high amounts of energy[1,25], the possibility of energy shortage is ever-present, particularly because food intake can be minimal or absent when an individual is responding to such threats[2–5]. Energetically, a living organism is a thermodynamically open system[26], so when responding to such threats in the presence of context-associated anorexia, the body depends on stored energy reserves in adipose tissue, muscle and the liver, and on other vital resources such as calcium stored in bone[1]. These energy reserves can be actively released via mechanisms that induce peripheral insulin resistance[27–29], a process that is central to energy distribution by causing the inhibition of energy-rich fuel storage. Similarly, calcium release can be triggered by the sympathetic nervous system and hypothalamic–pituitary–adrenal (HPA) axis acting via cortisol, or by an immune-activated increase in osteoclast activity[30–32], both of which are mechanisms that inhibit calcium storage.

In some potentially harmful situations (TABLE 1), the brain takes control to counter threats, coordinating skeletal muscles, heart, lungs and other organs to achieve this goal. Under these conditions, the brain can be thought of as being selfish, since there is no higher authority within the body to make decisions about energy distribution[29]. The brain demands energy in the form of glucose[33] using pathways specific to itself to induce insulin resistance[29].

Table 1 | **Adaptive responses to common major threats**

Threat	Immediate response(s)	Response systems
Predators	Fight or flight	Brain, SNS and HPA axis
Wounding and haemorrhage	Blood coagulation and blood pressure stabilization	• Brain, SNS, HPA axis and SNS-dependent RAA system • Standby system of blood coagulation
Food scarcity	Foraging	Brain, SNS and HPA axis
Thirst	Water-seeking behaviour	Brain, SNS, HPA axis and SNS-dependent RAA system
Cold	Warming-up and warmth-seeking behaviour	Brain, SNS and HPA axis
Heat	Sweating and cold-seeking behaviour	Brain and SNS
Infection	Immune response to infection	Immune system
Wounding	Inflammatory response	Immune system

HPA axis, hypothalamic–pituitary–adrenal axis; RAA system, renin–angiotensin–aldosterone system; SNS, sympathetic nervous system.

Similarly, the immune system can be thought of as being selfish during the response to infectious diseases or when healing infected wounds, since it takes the highest hierarchical rank in fighting these harmful situations. The immune system also demands energy using pathways specific to itself to induce insulin resistance[28,29,34]. Glucose is the favoured fuel because it can be rapidly metabolized into cellular energy in the form of ATP, and because it is useful under both normoxic and hypoxic conditions[16,17,19,35,36]. A pertinent example of immune system selfishness was provided by observations of the innate immune system of *Drosophila melanogaster*[37]. Similar to insulin resistance in mammals, in *D. melanogaster*, adenosine release is the main factor required for energy redistribution when the innate immune system is challenged[37].

The heart, lungs, kidneys and other organs could arguably also be called selfish because they can claim energy-rich substrates independently of the brain and immune system. Indeed, this statement is true for most organs in the body at the basal level to maintain organ function[25]; even a starving individual can maintain basal function of organs for a long time (~40–60 days)[38]. In contrast to the common major threats listed in TABLE 1, these other organs do not dominate the body because they do not take the highest hierarchical rank during a response. Sometimes these organs can dominate the body in states of chronic disease, for example during chronic heart failure or chronic kidney disease[39,40]; however, these scenarios are not considered to be important forces during evolutionary history. The placenta during pregnancy and mammary glands during lactation can also be considered selfish, but further discussion of these scenarios in healthy reproducing individuals is beyond the scope of this article. Importantly, the common major threats (TABLE 1) should be considered in parallel. For example, one cannot consider the threat of starvation on its own and thereby deduce that the brain is more selfish than the immune system. Depending on the type of threat, either the brain or the immune system dominates.

In the following section, a model is proposed that interrelates the brain and the immune system, in the form of mental memory and immunological memory, respectively, with the role of energy storage organs (FIG. 1).

Memory and energy regulation

Mental memory has evolved to minimize energy expenditure or, in other words, to protect energy stores, and is tuned to ancestral priorities when examined in the context of foraging and other paleolithic tasks[41]. Foraging for food requires an enormous amount of energy; for example, when studied on three randomly selected consecutive days, the Baka hunter-gatherers of southeast Cameroon spent more energy foraging for food than they obtained by eating what they found, creating a negative net energy balance[42]. Such a situation obviously cannot be maintained, and can be prevented by the memory of foraging strategies and of food location[41], thereby decreasing the time required for successful foraging in the wild[43].

In a similar way, immunological memory acts to spare energy reserves. On an individual's first encounter with a pathogen, the immune system takes 14 days to develop a protective response[44]. This process includes shaping the optimum immune response and the clonal expansion of T cells and B cells, which are highly energy-consuming processes[1,18,45]. Notably, the induction of this initial immune response usually happens during a critical period of infection-induced anorexia — that is, cessation of voluntary energy intake caused by the infection, an example of context-associated anorexia. The immune response to a second encounter with the same infectious agent is much faster (3–5 days), as clonal expansion of T cells and B cells can start at an early time-point when the microbial load is low[44]. Usually, this second encounter is associated with only minimal context-associated anorexia, so does not affect energy intake.

Both mental memory and immunological memory are directed towards foreign entities and self. Such activities as tool-making, the invention of language, writing manuscripts and the storage of data on computer hard disks clearly show the energetic advantage of mental memory and its respective storage tools. Likewise, learned tolerance of the immune system towards harmless autoantigens and innocuous foreign antigens on the body surfaces and effector memory against microorganisms is a memory function that spares energy reserves, suggesting that immunological memory has evolved to minimize energy expenditure.

Since the brain and the immune system are vitally important in orchestrating responses to external threats (TABLE 1), the memory functions of these two super-systems can be considered to have an ultimate role: the protection of energy stores. In addition, energy reserves in adipose tissue and skeletal muscles can be considered to represent a 'memory' of the energy state and health status of the body. From this point of view, three major organ systems endowed with a kind of memory exist that are instrumental to energy regulation: the brain, the immune system and the energy storage organs (adipose tissue and skeletal muscles) (FIG. 1a). Together, these organ systems comprise the normal or adaptive energy matrix, an evolutionarily positively selected network that ultimately links mental memory, immunological memory and energy memory with insulin resistance (FIG. 1b).

Necessarily, pathways exist that connect these three forms of memory. For example, the brain and the immune system are connected by cytokines, neurotransmitters and hormones to modulate each other[46]. Another classic example is leptin, which links adipose tissue and the brain[47], and also links adipose tissue with the immune system[48]. Similarly, afferent sensory nerve fibres exist in adipose tissue[49] and skeletal muscles[50], which connect these organs with the brain. The brain also uses vasopressin to regulate adipose tissue function[51]. Another example is IL-6, an important factor in skeletal muscles that interferes with the brain and the immune system[52], and that can also challenge the brain when produced by the activated immune system[46]. Macrophage-derived IL-6 can directly lead to lipolysis in adipocytes[53,54], and the resulting circulating free fatty acids, together with glycerol, stimulate hepatic gluconeogenesis in animal models[55]. Many more examples can be provided, but the general principal remains that such crosstalk serves to integrate the three memory organ systems.

Energy costs in inflammation

Immune cells require energy for housekeeping functions, as well as for a variety of specific tasks such as migration, cytokine synthesis, phagocytosis, antigen processing and other effector functions[45,56]. When immune cells are stimulated *in vitro*, they require ~25–30% more energy than quiescent immune cells[45]. This increase is mirrored in patients with active chronic inflammatory diseases, in whom the energy expenditure of peripheral blood mononuclear cells (PBMCs) increases to 25–30% above the normal level seen in healthy or immunosuppressed individuals[12]. Similar results are also seen in PBMCs from patients with acute infections[12].

An individual with a sedentary lifestyle who is 1.80 m tall and weighs 85 kg requires ~10,000 kJ daily (7,500 kJ of which daily requirement represents the resting metabolic rate)[25] (FIG. 2). The total amount of energy required by the entire immune system in an inactivated state is ~1,600 kJ daily, and this level can increase by 25% during mild activation to 2,000 kJ daily (REF. 1). This amount of energy expenditure is similar to that required by resting skeletal muscles and the brain[1]. During acute infections such as sepsis, the total energy expenditure of the immune system can increase by 30–60%[25,57], whereas chronic low-grade infections, such as chronic hepatitis C in patients with fully compensated liver disease, can lead to an increase in total energy expenditure of 10%[11], illustrating that even low-grade inflammation can lead to extra energy costs.

The range of the pro-inflammatory load in different situations is enormous. When serum IL-6 is measured by the same high-sensitivity ELISA as an estimate of inflammation, the pro-inflammatory load can range from 1–2 pg/ml in healthy young individuals[58] and 2–4 pg/ml in healthy elderly individuals[58], to 6–7 pg/ml in caregivers of patients with Alzheimer disease (a situation of psychological stress)[59], 10 pg/ml in patients with well-controlled RA[60], 100 pg/ml in newly diagnosed patients with RA, and can even climb as high as 10,000 pg/ml in patients with sepsis (R.H.S. unpublished observations). Thus, the level of energy expenditure experienced by an individual can be expected to differ depending on their pro-inflammatory load. The idea that energy expenditure is proportional to the degree of inflammation has been further exemplified in studies in healthy volunteers who were injected with recombinant IL-6 (REF. 13) or lipopolysaccharide[14]. In comparison with untreated active chronic inflammatory diseases or even sepsis, the increased levels of inflammation seen during ageing, in situations of psychological stress and in patients with chronic inflammatory diseases that are in remission are not considered to constitute situations of high energy expenditure. By contrast, in untreated active chronic inflammatory diseases, the overactive immune system dominates energy distribution within the body.

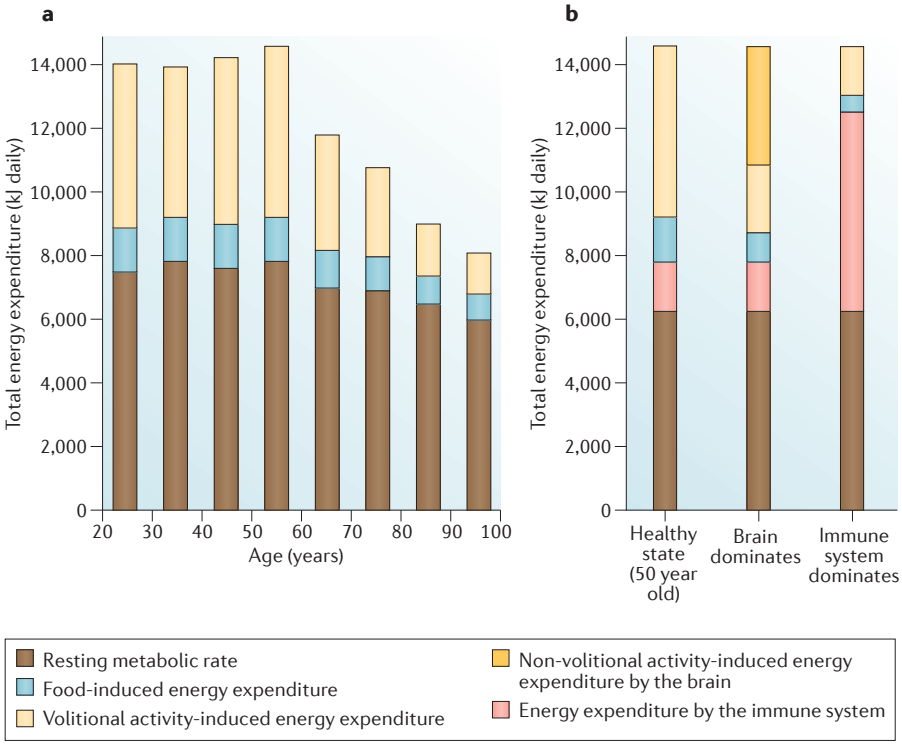

Figure 2 | **Average total energy expenditure for adults under various conditions. a** | Patterns of energy expenditure change as healthy individuals age[77]. The resting metabolic rate (or basal metabolic rate) is the amount of energy expended by an individual in the morning, 30 minutes after awakening, while lying in bed, relaxed, undertaking no physical activity and in thermoneutral conditions after one night of fasting. Food-induced energy expenditure, the thermogenic effect of food, is ~10% of the total energy expenditure under normal conditions. Activity-induced energy expenditure depends on the activity itself and whether it is volitional or non-volitional. **b** | An illustrative example of the average energy expenditure of a healthy 50 year-old individual; an individual with a 'dominant brain' (high levels of psychomotor activity and non-volitional physical activity); and an individual with a 'dominant immune system' (high levels of inflammatory activity and low levels of volitional physical activity). Notably, the energy expenditure of the immune system cannot yet be measured independently of the resting metabolic rate, so in this example the energy expenditure of the immune system is provided according to previous calculations[1].

Energy costs of brain activation

When an individual has a manic episode, psychomotor activity is high and energy expenditure is increased[61,62]. Similarly, energy expenditure can markedly increase in patients with dementia who have high levels of psychomotor activity, leading to cachexia[63]. These are special situations with high levels of psychomotor activity; however, when common forms of brain activation in the context of chronic inflammatory diseases or normal ageing are considered, pain, psychological stress, sleep alterations and anxiety can all become relevant. The effects of smoking should also be considered, as smoking can stimulate psychomotor activity[64,65]. These sequelae are not usually a major focus of treatment strategies, even though the associated psychomotor activity can induce extra energy costs.

An acute state of pain increases energy expenditure by up to 60%, as observed when painful electrical stimuli are applied to the abdominal skin of healthy individuals[66]. In this setting, energy expenditure parallels heart rate, blood catecholamine levels and levels of serum cortisol; thus, pain can increase insulin resistance[67]. Pain-induced increases in energy expenditure are fuelled by many energy sources, although the largest increase is seen in glucose utilization[66]. Similarly, postoperative removal of analgesic medication (resembling a more chronic pain situation over several days), can lead to a high level of pain and an increase in energy expenditure of 15%[68]. For ethical reasons, long-term experimental studies on patients with chronic pain are not possible, but the available evidence indicates that pain entails extra energy costs.

During a period of acute psychological stress, such as during a laboratory stress test, the energy supply to the human brain increases by 12%[69]. Directly following a 10-minute stress experiment, the general energy intake increased by 26% of the normal daily requirements of the brain (570 kJ), and the test induced a state of "cerebral insulin resistance" (REF. 69). Neurodegenerative diseases with high levels of physical activity, such as Parkinson disease, Alzheimer disease and Huntington disease, can be chronically stressful and, in the case of Huntington disease, can increase energy expenditure up to 20%[63]. Thus, the added stress caused by diseases such as dementia might result in extra energy costs. Similarly, the added stress of providing care for a beloved family member increases energy expenditure by 20%[70]. Psychological stress seems to be expensive in terms of energy consumption.

Many of the aforementioned conditions also decrease the quality of an individual's sleep. Normal sleep is accompanied by a 30% decrease in energy expenditure during sleeping time[71], whereas reduced sleeping time and sleep quality can increase energy expenditure by 5–15%[72,73]. In the extreme situation of obstructive sleep apnoea, energy expenditure can increase by as much as 30%[74]. In this situation, it is as if the individual has not reduced energy expenditure at all while asleep. Importantly, energy uptake following sleep disruption (especially at night after an evening meal) can exceed sleep alteration-dependent energy expenditure, resulting in a positive net energy balance[72]. This situation is particularly relevant in obesity when levels of physical activity are low.

Anxiety can also increase energy expenditure, as shown in a study in which 79 male students were investigated for state and trait anxiety[75]. Notably, students who scored highly for anxiety (highest quartile) had a 10% increase in energy expenditure compared with students with a low anxiety score (lowest quartile)[75]. Furthermore, smoking not only increases the level of activity in the central and peripheral nervous systems, but also affects energy expenditure. In a 2014 study, total energy expenditure was increased by ~15% in men who smoked more than six cigarettes per day compared with men who had never smoked[64]. In summary, typical situations associated with psychomotor activation, which can happen in patients with chronic inflammatory diseases or during ageing, can cause extra energy costs.

Non-volitional energy costs

Extra energy expenditure caused by the immune system in the form of inflammation, or by the brain in the form of pain, psychological stress, sleep alterations and anxiety, can create an energy shortage. Reduced supplies of energy-rich fuels are particularly pertinent in situations involving context-associated anorexia[2–5,76]. These extra energy costs are deemed to be non-volitional or unintended. During the process of ageing or in a patient with a chronic inflammatory disease that is in remission, extra energy costs are due to low-grade inflammation[11] and, if present, non-volitional brain-derived energy expenditure. High levels of immune system-induced energy expenditure, such as those seen in patients with newly diagnosed chronic inflammatory diseases or in patients with chronic inflammatory diseases during a flare, can also often be accompanied by brain-derived extra energy costs, in particular due to pain and altered sleep.

Although, under normal conditions in a healthy individual, volitional activity-induced energy expenditure slowly decreases during ageing[77] (FIG. 2a), volitional activity can decrease immediately in a situation in which the brain or the immune system suddenly becomes dominant (FIG. 2b). This change can occur in young or old individuals, but is more problematic in the elderly owing to the natural reduction in volitional energy expenditure and energy intake that occurs as an individual ages[77] (FIG. 2a).

The non-volitional energy expenditure caused by inflammation can be very high[25,57], and a situation of acute energy shortage cannot last for longer than 19–43 days before stored energy reserves are depleted[38]. This time-frame is known as the 'complete energy consumption time' in the presence of complete anorexia, as calculated for an individual infected with influenza virus[32,38]. Physical considerations of energy follow simple mathematical rules of summation and subtraction, as shown in an example of a healthy individual of 50 years of age[77] (FIG. 2b). Using the same example, BOX 1

Box 1 | Calculation of total energy expenditure with non-volitional energy costs

Total energy expenditure (EE in the following equations) is a mathematical sum comprising many parts:

Total EE = resting metabolic rate + food-induced thermogenesis + volitional physical and mental EE

In a situation in which there are high levels of non-volitional activity in the immune system or brain, the equation must be modified:

Total EE = resting metabolic rate + food-induced thermogenesis + volitional physical and mental EE + non-volitional EE

Assuming that non-volitional energy expenditure occurs in the presence of normal levels of total energy expenditure and resting metabolic rate, with a reduced food-induced thermogenesis due to context-associated anorexia[2–5], there will be a reduction in volitional physical and mental activity. In this situation, a reduced portion of total energy is available for volitional activities and, additionally, context-induced anorexia hinders or prevents adequate energy intake to compensate for this loss.

Table 2 | **Activities causing a non-volitional increase in daily energy expenditure**

Non-volitional activity	Extra energy costs*	Refs
Inflammation	25–60%‡	1,57
Chronic low-grade infection§	10%	11
Acute pain	up to 60%	66
Chronic pain	15%	68
Psychological stress	up to 30%	63,69,70
Sleep alterations	up to 30%	72–74
Anxiety	up to 10%	75
Heavy smoking	up to 15%	64

*Extra energy costs are relative to total energy costs in healthy individuals, and are given as a percentage of the basal or total energy expenditure. ‡Range spans mild activation to sepsis. §Such as hepatitis C infection.

explains how a reduced proportion of total energy is available for volitional activities when non-volitional activities occur (TABLE 2).

Non-volitional activities causing energy expenditure cannot be viewed independently, as the causes of non-volitional energy expenditure often accompany each other (for instance, pain is often accompanied by disturbed sleep and pain represents a form of psychological stress). The cumulative percentage increase of extra energy expenditure will therefore not be the sum of the individual percentages mentioned in TABLE 2. Nevertheless, when accompanied by context-induced anorexia, the increased extra energy expenditure for such non-volitional activities should lead to a shortage of energy available for partaking in volitional activities. This energy shortage can lead to undesired sequelae resulting from reduced physical and mental activity[78–80].

Consequences of energy shortage

Although this article cannot explain the growing field of evolutionary medicine in detail (see REFS 15,23,24), one can summarize that, during evolution, homeostatic networks were positively selected for use in short-lived acute energy-consuming responses rather than for use in long-standing chronic inflammatory diseases[15]. Long-standing responses of the super-systems (for example, courtship behaviour, mental memory and immunological memory) were positively selected to support reproduction and to protect energy stores. Although several genetic risk factors that favour the development of chronic inflammatory diseases have been identified in these super-systems, such as *HLA-DR4* as a risk factor for RA[81], these factors are unlikely to have been positively selected for their ability to induce or specifically aggravate such diseases. Instead, these factors increase reproductive fitness in individuals of pre-reproductive and reproductive ages (for example, *HLA-DR4* offers some protection against Dengue haemorrhagic fever[82]), with the resulting fitness benefits at reproductive age expected to be higher than the fitness costs at post-reproductive age[83] (for example, the development of RA). Overall, an individual's lifetime reproductive success will be increased by traits such as this, thereby providing an explanation for why factors that favour chronic inflammatory diseases have not been out-selected during evolution. In fact, under specific environmental conditions, such factors might even have been positively selected under a different somatic context (such as for fitness during reproductive age but not for post-reproductive individuals)[15,83].

The signs and symptoms that often accompany chronic inflammatory diseases are elements of evolutionarily positively selected programmes. These programmes are in no way unfavourable when used for a short period of time; they are adaptive, can be used in various situations when energy is scarce and can help to build the adaptive energy matrix to protect energy stores (FIG. 3a). Utilizing these programmes can provide the body with a sufficient supply of energy-rich fuels and other nutrients, such as calcium, in the presence of threats and in situations in which context-induced anorexia occurs[32].

In previous articles[1,15,29,32,38], I indicated that the signs and symptoms mentioned at the beginning of the article (see Supplementary information S1 (table)) are merely the unfavourable sequelae of chronic disease, but this is not correct. Instead, I propose a new model, in which these signs and symptoms represent positively selected and highly favourable coping mechanisms used by the body in situations of short-lived energy undersupply. These adaptive responses are not designed to be used for long periods of time; in fact, long-term use of these mechanisms will necessarily lead to the development of a maladaptive energy matrix, with concomitant loss of energy reserves. If an energy shortage cannot be overcome, volitional physical and mental energy expenditure markedly decrease for a prolonged period of time (FIG. 3b). The long-term consequences of reduced physical and mental activity include cardiovascular diseases[84], metabolic diseases and obesity, cognitive dysfunction and pain[85,86], neuro-degenerative disease[87,88], psychiatric diseases[89], frailty[90] and other chronic diseases such as cancer[91]. These conditions all constitute risk factors for early death.

Conclusions

In this Perspectives article, I have provided the reader with a framework that accounts for increased energy expenditure caused by psychomotor activity in chronic

Glossary

Context-associated anorexia
Anorexia that is dependent on a particular circumstance, such as sickness behaviour during an infection, mental activation in bipolar disorder or age-related anorexia.

Insulin resistance
A condition of low insulin sensitivity with marked changes to the insulin receptor and to downstream signalling pathways; because insulin is responsible for the storage of glucose and free fatty acids, a lower insulin sensitivity leads to reduced energy storage and increased levels of energy-rich fuels in the circulation.

Pro-inflammatory load
A high level of systemic activity in the immune system, as measured by an increased erythrocyte sedimentation rate, or increased levels of serum C-reactive protein or serum IL-6.

Psychomotor activity
Activity induced by the brain that leads to activation of the skeletal muscles and the heart.

Super-systems
Integrative systems at the top level of homeostatic regulation of the body; examples include the nervous, endocrine and immune systems.

Thermodynamically open system
Systems, such as the human body, that can take up and lose energy, mainly in the form of heat.

State and trait anxiety
State anxiety is how a person is feeling at the time of a perceived threat, whereas trait anxiety is the enduring disposition to feel stress, worry and discomfort.

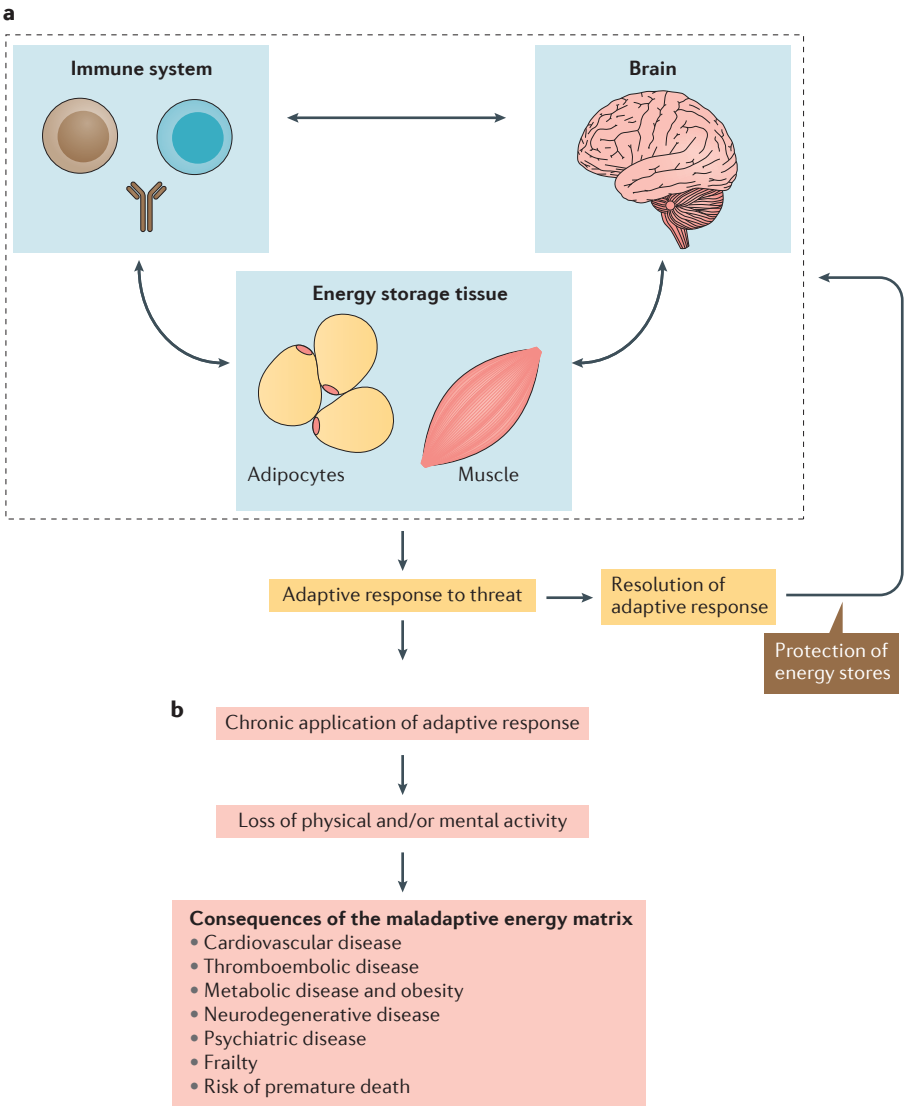

Figure 3 | **The adaptive and maladaptive energy matrices. a** | The adaptive energy matrix represents the evolutionarily positively selected normal or adaptive response to a threat. The brain, immune system and energy storage organs (adipose tissue and skeletal muscles) communicate with each other and induce adaptive responses depending on the prevailing short-term threat. Such adaptive responses are necessary to protect energy stores. **b** | Short-lived, adaptive responses to threats were not positively selected for use in chronic inflammatory diseases or during ageing, so prolonged use of these responses falls outside of adaptive norms. Chronic application of these responses transforms the adaptive energy matrix into the maladaptive energy matrix, leading to disease and premature death. Central to the health problems caused by the maladaptive energy matrix is a loss of physical and mental activity in an individual.

inflammatory diseases and during ageing. Previous models mainly focused on increased energy expenditure caused by a highly active immune system[1], but did not account for energy expenditure caused by psychomotor activity, making these previous models applicable to patients with active chronic inflammatory diseases, but not to patients with chronic inflammatory diseases that are in remission or elderly individuals. In individuals from the last two groups, the proportion of energy expended by the immune system is small, so immune-derived energy shortage cannot explain the appearance of adaptive programmes in these individuals (see Supplementary information S1 (table)): immune system-independent energy expenditure must be involved.

The proposed framework is based on the idea that energy shortage in these groups of individuals depends on increasing energy expenditure caused by high levels of psychomotor activity. For example, pain, psychological stress, sleep alterations and anxiety increase energy expenditure. This framework includes aspects of evolutionary medicine (concepts of selfishness, memory crosstalk and short-term adaptive programmes), energy regulation (energy expenditure in inflammation, energy expenditure in brain activation and the idea of three memory organ systems relevant to energy protection) and neuroendocrine adaptive programmes that are positively selected to overcome short-lived activation of the immune system or brain. The proposed model demonstrates the concept of non-volitional energy expenditure, which reduces physical and mental activity, ultimately leading to an increased risk of cardiovascular diseases, metabolic diseases and obesity, cognitive dysfunction and pain, neurodegenerative diseases, psychiatric diseases, frailty and cancer (FIG. 3).

In the future, studies should aim to quantitatively detect sequelae of chronic inflammatory diseases (see Supplementary information S1 (table)) and to measure energy expenditure, energy intake and physical activity using a variety of techniques. The current goal of achieving disease remission is not ambitious enough. Physicians and pharmaceutical companies should take the next step towards an integrative approach and conduct randomized controlled trials that look beyond immunosuppression.

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