SYSTEMIC LUPUS ERYTHEMATOSUS

Keratinocytes: wolves in sheep's clothing

> type I IFN activity was enriched in the skin of patients with SLE and at-risk individuals

Abundant evidence highlights the important role of type I interferons (IFNs) in the pathogenesis of systemic lupus erythematosus (SLE). However, the cellular source of type I IFNs and their regulation in disease initiation is unclear. New findings cast doubt on the notion that plasmacytoid dendritic cells (pDCs) are key contributors to excessive type I IFN production in SLE, and instead implicate human keratinocytes as a source of type I IFN in SLE, including in the early stages of autoimmunity.

To understand the phenotype and function of pDCs in preclinical autoimmunity and in disease, the researchers analysed peripheral blood mononuclear cells from healthy individuals, patients with SLE, patients with primary Sjögren syndrome (pSS) and 'at-risk' individuals (defined as anti-nuclear antibody-positive individuals who were treatment-naive and had no more than one clinical criterion for SLE, with a symptom duration of less than 12 months).

The patients with SLE or pSS and the at-risk individuals had decreased numbers of pDCs compared with healthy individuals, which was independent of disease activity and immunosuppressive treatment. Despite this reduction, all three patient groups had increased type I IFN activity in their blood that was unrelated to the reduction in pDC number.

Further analysis showed that the function of the pDCs was impaired in patients with SLE or pSS and in at-risk individuals. Compared with pDCs from healthy individuals, the pDCs from these individuals produced less cytokines and had a reduced capacity to stimulate T cell activation and proliferatation. Again, this reduction in function was unrelated to disease activity, therapy or IFN activity.

"Previous researchers had suspected that reduced numbers of pDCs in the blood of patients with SLE was due to pDC migration into inflamed tissues. However, a key aspect of our work is that the same numeric and functional defect was seen in at-risk individuals who never developed clinical autoimmunity or tissue inflammation," reports the lead author Edward Vital.

RNA sequencing analysis of purified pDCs from healthy individuals, patients with SLE or at-risk individuals found that the pDCs clustered according to the expression of IFN-stimulated genes (ISGs), rather than according to clinical phenotype. Hence, the researchers first assigned each sample an IFN score (on the basis of the expression of a selection of ISGs) and grouped the samples into two subgroups: an IFN^{low} and an IFN^{high} subgroup.

Given that the function of pDCs was impaired in patients with SLE irrespective of IFN activity, the researchers examined the transcripts that were differentially expressed in pDCs from patients with SLE in both the IFN^{high} and IFN^{low} subgroups, compared with pDCs from healthy individuals, narrowing the list down to 80 differentially expressed transcripts. Among these transcripts were genes involved in cellular senescence and stress.

Further in vitro experiments found that pDCs from patients with SLE had shorter telomere lengths compared with pDCs from healthy individuals, and that mild oxidative stress could inhibit the ability of healthy pDCs to produce IFNα, further implicating these two processes in the loss of function of pDCs in preclinical SLE.

Given that pDCs are unlikely to be the source of aberrant type I IFN production in SLE, and that type I IFN activity in the blood was associated with mucocutaneous disease activity, the researchers examined paired skin and blood samples. Notably, type I IFN activity was enriched in the skin of patients with SLE and at-risk individuals (being 5,000 times higher in the skin of some patients than in healthy individuals).

Further analysis identified keratinocytes as the source of this type I IFN activity. Even at baseline, keratinocytes from the skin of at risk individuals or patients with SLE expressed IFNκ (unlike in healthy individuals), and this expression further increased following in vitro stimulation.

"These findings suggest keratinocytes as a source of type I IFN in autoimmune disease, and raise questions about the relative contribution of pDCs to the well described type I IFN signature in autoimmunity," says Timothy Niewold, an expert on type I IFNs in SLE who was not involved in the study. "It will be fascinating to understand the cause of IFNκ production in keratinocytes, and why this production occurs in patients with autoimmune or incomplete autoimmune disease."

Jessica McHugh

Credit: Planet Flem/DigitalVision Vectors

ORIGINAL ARTICLE Psarras, A. et al. Functionally impaired plasmacytoid dendritic cells and non-haematopoietic sources of type I interferon characterize human autoimmunity. *Nat. Commun.* **11**, 6149 (2020)

RHEUMATOID ARTHRITIS

Regulatory eosinophils to the rescue

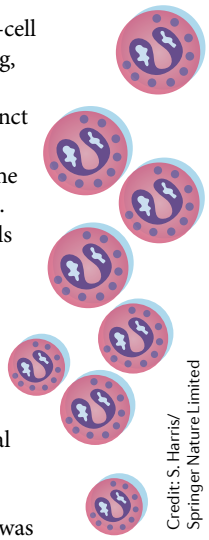
Eosinophils are typically associated with type 2 immunity and inflammatory conditions such as asthma and allergies; however, they can also produce anti-inflammatory molecules, and previous studies have linked type 2 immune responses and eosinophils to arthritis resolution. The results of a new study reveal how a subset of eosinophils can regulate arthritis resolution and provide evidence for a beneficial effect of certain types of asthma on arthritis.

"In this study, we identified for the first time an immune-regulatory eosinophil subset in the synovial tissue of mice and humans," states corresponding author Aline Bozec. "Strikingly, the signature of pro-resolving synovial eosinophils is completely distinct from the inflammatory eosinophils that arise in the lungs during asthma."

Bozec and colleagues began by establishing that mice with type 2 allergic asthma (characterized by eosinophilia) were able to resolve K/B×N serum transfer-induced arthritis more quickly than mice without asthma. This rapid disease resolution was associated with an increased number of eosinophils in the joints, and could not be achieved when asthma and arthritis were induced in mice that lacked eosinophils.

> "rapid disease resolution was associated with an increased number of eosinophils in the joints"

Using single-cell RNA sequencing, the researchers discovered distinct phenotypes of eosinophils in the lungs and joints. Lung eosinophils from mice with asthma and arthritis had a classic pro-inflammatory phenotype, whereas synovial eosinophils had a pro-resolving phenotype that was characterized by the production of lipid resolvins.



Credit: S. Harris/ Springer Nature Limited

Mechanistically, Bozec and colleagues showed that regulatory eosinophils expand in response to IL-5 produced by type 2 innate lymphoid cells in the lungs, and that they elicit their pro-resolving effects in the joints by stimulating alternatively activated macrophages.

Eosinophils have similar roles in mice and humans, so these cells might also be important for rheumatoid arthritis (RA); indeed, patients with RA in remission had increased numbers of regulatory eosinophils in their joints compared with patients with active RA. Interestingly, treatment of asthma with an anti-IL-5 antibody induced RA flares in patients who had both asthma and RA that was previously in remission, suggesting an important role for IL-5, and potentially for regulatory eosinophils, in RA resolution.

Joanna Clarke

ORIGINAL ARTICLE Andreev, D. et al. Regulatory eosinophils induce the resolution of experimental arthritis and appear in remission state of human rheumatoid arthritis. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-218902 (2020)

LUPUS NEPHRITIS

Targeting Kv1.3 channels on T cells

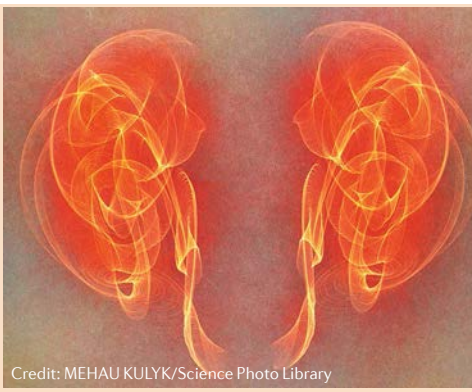
Kidney infiltration of activated memory T (T_M) cells contributes to the pathogenesis of lupus nephritis (LN). These cells express high levels of voltage-gated Kv1.3 potassium channels, which are important regulators of T cell function owing to their role in control of Ca^{2+} influx. A new study reports that selectively targeting Kv1.3 on T_M cells using nanoparticles could potentially be beneficial in LN.

The researchers found increased infiltration of $CD4^+$ and $CD8^+$ T cells in kidney biopsy samples from patients with LN or diabetic kidney disease (DKD) compared with normal kidney samples. Moreover, the LN samples had a higher ratio of $CD8^+$ to $CD4^+$ T cells and a fourfold higher density of $CD8^+CD45RO^+$ T_M cells than the DKD samples.

Immunofluorescence staining indicated higher expression of Kv1.3, the cytotoxic protease granzyme B and the proliferation marker Ki-67 in kidney-infiltrating $CD8^+$ T cells from patients with LN than in those from normal kidneys. "These results showed that high Kv1.3 expression in LN [kidney-infiltrating T cells] occurs with increased cytotoxicity and cell proliferation," say the researchers. "Therefore, a therapy that blocks Kv1.3 channels in T_M cells of patients with LN could reduce the activity of [these T cells]."

> "... targeted knockdown of Kv1.3 using nanoparticles might be of therapeutic value in [lupus nephritis]"

To test this hypothesis, the researchers used lipid nanovesicles containing small interfering RNA against Kv1.3 and coated with a monoclonal antibody against CD45RO to selectively target T_M cells. They report that incubation of T cells from patients with LN with these nanoparticles reduced T cell expression of CD40L and IFNγ, which contribute to the pathogenesis of LN. Similarly, pretreatment of peripheral blood mononuclear cells with Kv1.3 nanoparticles before engraftment in a humanized mouse model of LN resulted in a reduction in the expression of CD40L and IFNγ on splenocytes at day 7 and improved the survival of these mice.



Credit: MEHAU KULYK/Science Photo Library

The researchers conclude that targeted knockdown of Kv1.3 using nanoparticles might be of therapeutic value in LN. However, they caution that long-term studies in mice with established pathology are required to further investigate this strategy.

Ellen F. Carney

This article is modified from the original in *Nat. Rev. Nephrol.* (https://doi.org/10.1038/s41581-020-00387-y).

ORIGINAL ARTICLE Khodoun, M. et al. Targeted knockdown of Kv1.3 channels in T lymphocytes corrects the disease manifestations associated with systemic lupus erythematosus. *Sci. Adv.* **6**, eabd1471 (2020)

EPIDEMIOLOGY IN 2020

Spectrum and impact of checkpoint inhibitor-induced irAEs

Laura C. Cappelli and Clifton O. Bingham III

Immune checkpoint inhibitors, which are used to treat many types of cancer, can cause syndromes similar to rheumatic diseases known as immune-related adverse events (irAEs). In 2020, several studies illustrated the clinical heterogeneity of rheumatic irAEs and highlighted their substantial effect on morbidity and mortality.

Immune checkpoint inhibitors (ICIs), the most commonly used class of cancer immunotherapy, can cause immune-related adverse events (irAEs). IrAEs are inflammatory syndromes affecting almost any organ system that often share similarities with autoimmune inflammatory diseases. The spectrum of irAEs with similar manifestations to autoimmune rheumatic disease includes inflammatory arthritis, sicca syndrome, polymyalgia rheumatica (PMR), myositis, vasculitis, systemic sclerosis (SSc, also known as scleroderma) and several other syndromes[1]. Although rheumatic irAEs were not well-recognized in initial clinical trials of ICI therapy, they are now increasingly noted, and several studies published within the past year highlight the range of rheumatic events induced by ICI therapy, their effects on patients with cancer and the outcomes of irAE treatment[2–4] (FIG. 1).

One potential reason that rheumatic irAEs were initially studied less frequently than other irAEs, such as colitis or pneumonitis, was the perception that they were associated with lower rates of morbidity and mortality. A 2020 study by Allenbach et al.[2] utilized the World Health Organization (WHO) pharmacovigilance database to evaluate rheumatic irAEs, with disproportionality analysis performed to identify whether drug-induced rheumatic events were more common in ICI-treated patients as compared with patients in the entire database. A total of 1,288 rheumatic irAEs were identified. The highest reporting odds ratio (ROR) for a rheumatic irAE was for PMR (ROR 14.6, 95% CI 11.6–18.4), followed by sarcoidosis (ROR 9.6, 95% CI 7.9–11.9) and Sjögren syndrome (ROR 6.9, 95% CI 5.2–9.2). The full range of rheumatic irAEs over-reported in ICI-treated patients in the WHO database included myositis, arthritis and SSc. Notably, systemic lupus erythematosus and mixed connective tissue disorder were not over-reported, consistent with prior observations[5]. Rheumatic irAEs were more frequently reported in patients who received a combination of CTLA4 and PD1 blockers as opposed to monotherapy with either class of agent alone. Myositis had the highest mortality rate of all the rheumatic irAEs (24%); other irAEs had mortality rates of 0–6.7%. This high rate of mortality for myositis was attributable at least in part to those patients with concurrent myocarditis or myasthenia gravis, who had even higher mortality rates (56.7% and 27.9%, respectively). Myositis also had the shortest time to onset from initiation of ICI therapy (median 31 days). The results of this study emphasize the severity of ICI-induced myositis, particularly if associated with myocarditis or myasthenia gravis. Clinicians should be aware of this early and severe irAE.

Another 2020 study that focused on the full spectrum of rheumatic irAEs was a multicentre study by Roberts et al.[3], which reported data from May 2018 onward from ten academic rheumatology centres across Canada. A total of 136 rheumatic irAEs were identified; the most common of which was symmetric polyarthritis, followed by PMR-like symptoms and sicca syndrome. Most patients had grade 1 (mild) or grade 2 (moderate) irAEs according to the Common Terminology Criteria for Adverse Events (CTCAE). About two-thirds of the patients required temporary holding or discontinuation of ICI therapy. The majority (65%) of patients received oral glucocorticoids but only one-third of these patients achieved a complete response to steroid treatment. DMARDs used included methotrexate, hydroxychloroquine, mycophenolate mofetil, sulfasalazine and leflunomide. Eight patients were treated with TNF inhibitors, and one patient received rituximab. Most patients in the study had a complete or partial response of their cancer to ICI therapy. Among patients treated for their irAE, this tumour response was maintained or improved in all except 7.7%, whose tumours worsened. The findings from this study confirm that inflammatory arthritis is the most frequent irAE encountered by rheumatologists and also show that most patients did not have a worsening of their tumours despite treatment of the irAE with corticosteroids and/or DMARDs.

In addition to the impact of irAEs on mortality and tumour response, their effects on patients' quality of life are beginning to be appreciated. In 2020, the first qualitative study of a rheumatic irAE, inflammatory arthritis, was published. In this study, researchers interviewed 14 patients with inflammatory arthritis attributable to ICI therapy[4]. Participants noted a delay in the diagnosis of arthritis owing to a lack of awareness, of both patients and health-care providers, of arthritis being

Key advances

- Immune checkpoint inhibitor (ICI)-induced myositis has a high mortality rate, particularly when associated with myocarditis and myasthenia gravis, and can happen shortly after ICI initiation[2].
- Rheumatic immune-related adverse events (irAEs) of all kinds necessitate systemic immunosuppression, and for most patients in one multicentre study, tumour response did not worsen after treatment of these irAEs[3].
- Patients with inflammatory arthritis attributable to ICI therapy experience substantial emotional and functional effects, outcomes that could be improved with increased awareness, multidisciplinary care and increased social support[4].

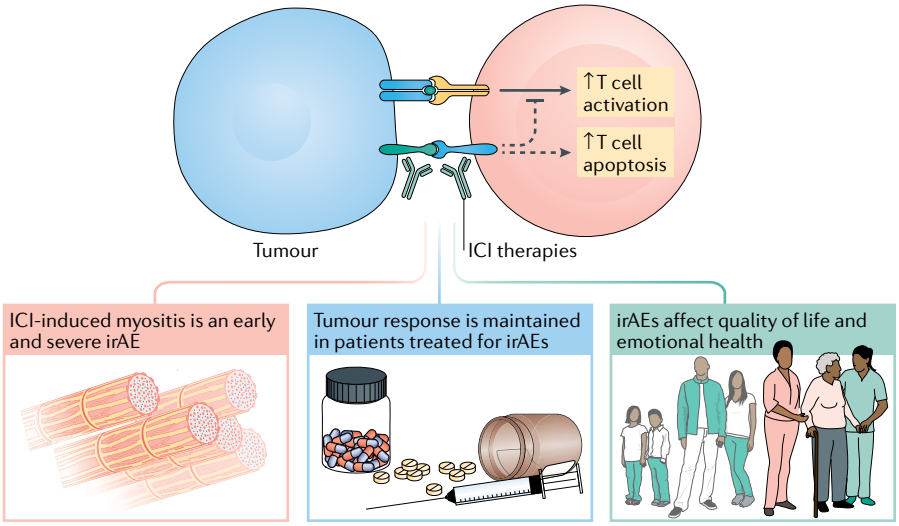


Fig. 1 | **New insights into the occurrence and effects of ICI-induced irAEs.** Studies published in 2020 highlight the clinical spectrum of immune checkpoint inhibitor (ICI)-induced immune-related adverse events (irAEs) and their effects on mortality, tumour response and patients' quality of life.

an irAE from ICI therapy. A somewhat surprising finding was that the emotional and quality of life effects of inflammatory arthritis were as severe or worse than those of other irAEs or the underlying cancer diagnosis. Patients also felt less supported by family and friends through their arthritis diagnosis as compared with their cancer diagnosis. Finally, decision-making proved complicated for patients with ICI-induced inflammatory arthritis, owing to fear of cancer returning or advancing and uncertainty about their cancer prognosis. This fear influenced whether patients reported symptoms of inflammatory arthritis to their physicians, whether they wanted to continue ICI therapy and whether they started immunosuppressive medications to treat the arthritis. This study highlights the difficult decisions patients with rheumatic irAEs must make; this difficulty is enhanced by the lack of evidence regarding the treatment of rheumatic irAEs.

Adding to these epidemiological and clinical observations, several other studies have evaluated the imaging characteristics of musculoskeletal irAEs. In a 2020 study of eight patients with inflammatory arthritis attributable to ICI therapy[6], MRI examinations identified tenosynovitis and bone marrow oedema in small joints, and joint effusions and synovial thickening in larger joints. Notably, early erosive disease was evident, occurring as soon as 4 weeks after the onset of symptoms in one patient. These findings are similar to those from past studies, in which patients with ICI-induced inflammatory arthritis were evaluated using ultrasonography[7]. Another MRI study examined ten patients who developed more general musculoskeletal complaints (as opposed to inflammatory arthritis specifically) during ICI therapy[8]. Three main patterns were evident on MRI: prominent joint involvement, prominent periarticular involvement and myofasciitis. Patients with periarticular involvement were almost a hybrid of the other groups in that they had tenosynovitis and myositis and/or fasciitis in tissues surrounding the affected joints. The finding of myofasciitis in several patients with musculoskeletal pain suggests that the affected tissue might not always be the synovium or joint structures. This finding has implications for understanding the pathogenesis of and risk factors for irAEs, as the underlying biology could differ between patients with prominent synovitis and those with prominent myofasciitis. This study, like others, found a higher rate of tumour response in ICI-treated patients who developed musculoskeletal compared with those who did not (50% versus 12.5%, $P=0.0016$).

These and other studies have opened exciting avenues of research in the field of rheumatic irAEs associated with ICI therapy. Further investigation into the heterogeneity of rheumatic irAEs is warranted as it could reveal biologically relevant subgroups for analysis. Larger imaging studies using MRI and ultrasonography could tell us more about patterns of involvement and define the relevant tissue for further laboratory analysis. Treatment studies should focus both on corticosteroid dosing and optimal use of steroid-sparing agents. Prospective studies with long-term follow-up will increase the confidence of clinicians counseling patients receiving ICI therapy who develop irAEs about the effects of immunosuppression on their tumour response.

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https://doi.org/10.1038/s41584-020-00546-2

1. Kostine, M., Truchetet, M. E. & Schaeverbeke, T. Clinical characteristics of rheumatic syndromes associated with checkpoint inhibitors therapy. *Rheumatology* **58** (Suppl. 7), vii68–vii74 (2019).
2. Allenbach, Y. et al. Immune checkpoint inhibitor-induced myositis, the earliest and most lethal complication among rheumatic and musculoskeletal toxicities. *Autoimmun. Rev.* **19**, 102586 (2020).
3. Roberts, J. et al. Rheumatic immune-related adverse events associated with cancer immunotherapy: a nationwide multi-center cohort. *Autoimmun. Rev.* **19**, 102595 (2020).
4. Cappelli, L. C., Grieb, S. M., Shah, A. A., Bingham, C. O. 3rd & Orbai, A. M. Immune checkpoint inhibitor-induced inflammatory arthritis: a qualitative study identifying unmet patient needs and care gaps. *BMC Rheumatol.* **4**, 32 (2020).
5. Calabrese, L. H., Calabrese, C. & Cappelli, L. C. Rheumatic immune-related adverse events from cancer immunotherapy. *Nat. Rev. Rheumatol.* **14**, 569–579 (2018).
6. Subedi, A. et al. Use of magnetic resonance imaging to identify immune checkpoint inhibitor-induced inflammatory arthritis. *JAMA Netw. Open* **3**, e200032 (2020).
7. Albayda, J., Dein, E., Shah, A. A., Bingham, C. O. 3rd & Cappelli, L. Sonographic findings in inflammatory arthritis secondary to immune checkpoint inhibition: a case series. *ACR Open Rheum.* **1**, 303–307 (2019).
8. Daoussis, D. et al. An MRI study of immune checkpoint inhibitor-induced musculoskeletal manifestations myofasciitis is the prominent imaging finding. *Rheumatology* **59**, 1041–1050 (2020).

Competing interests

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COVID-19 IN 2020

Rheumatic disease and COVID-19: epidemiology and outcomes

Kimme L. Hyrich and Pedro M. Machado

Since the outset of the COVID-19 pandemic, numerous risk factors for severe disease have been identified. Whether patients with rheumatic diseases, especially those receiving DMARDs, are at an increased risk of SARS-CoV-2 infection or severe COVID-19 disease remains unclear, although epidemiological studies are providing some insight.

The COVID-19 pandemic, caused by SARS-CoV-2 infection, has seen over 71 million confirmed cases and over 1.6 million deaths worldwide recorded up to 15 December 2020, although the true number of cases worldwide is unknown[1]. For most people, COVID-19 will cause a mild-to-moderate flu-like illness characterized by fever, cough, and loss of taste and smell, among other symptoms; for some patients, however, the disease takes a severe and aggressive form, requiring hospitalization and ventilatory support, and potentially results in death. Whether or not patients with pre-existing immune-mediated inflammatory diseases (IMIDs), such as rheumatic diseases, are at an increased risk of SARS-CoV-2 infection or of severe COVID-19 outcomes remains unclear. Over the past 6 months, an unprecedented number of case series and reports of COVID-19 in patients with rheumatic diseases have been published; in this Year in Review commentary we highlight three of the larger studies published over this period of the pandemic[2–4], which have advanced our knowledge of the risk of COVID-19 in this population (FIG. 1).

Using data from the new OpenSAFELY electronic platform, holding the primary care health records of ~17 million adults in England, Williamson et al.[2] examined factors associated with 10,926 COVID-19-related deaths (0.06% of the study population) over the first 3 months of the pandemic. They observed that people with a diagnosis of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or psoriasis, analysed as a combined group (5.1% of the study population), were (slightly) more likely to die in relation to COVID-19 compared to people without one of these diagnoses; this finding persisted after allowing for any variation in age, sex, ethnicity, social deprivation and the presence of other chronic health conditions (adjusted HR 1.19; 95% CI 1.11–1.27). This observation is important but is limited by the lack of more specific knowledge of the diseases at the individual level, a common limitation of primary care data. Rheumatic diseases are highly heterogeneous, and thus information on current levels of disease activity, specific disease-related comorbidities and, most critically, the use of glucocorticoids and/or DMARDs, all of which are themselves risk factors for serious infection[5], is needed to further understand what is driving this increased risk of COVID-19-related death. The investigators also did not have access to data on the prevalence of SARS-CoV-2 infection in the population, so it is not known whether patients with these conditions are at increased risk of infection or of dying if infected. Although tempting, causal interpretations of the OpenSAFELY findings should be avoided.

The potential role of DMARDs in the presentation, severity and even management of COVID-19 has received considerable attention. At the outset of the pandemic, hydroxychloroquine was touted as both a preventive and therapeutic treatment for COVID-19, but subsequent clinical trials have not found any benefit[6]. Many cytokines, such as TNF and IL-6, are involved in both the physiological response as well as the pathological response (for example, the 'cytokine storm') seen with COVID-19 (REF.[7]). Cytokine inhibitors used in rheumatic diseases are therefore of interest, in terms of whether they are effective treatments for severe COVID-19 and whether their chronic use in patients with rheumatic diseases might alter the course of infection. However, the role of a cytokine storm in COVID-19-induced organ dysfunction has been questioned, as the extent of cytokinaemia in cases of severe and critical COVID-19 is less than that seen in other disorders associated with elevated cytokine production, such as chimeric antigen receptor (CAR) T cell-induced cytokine release syndrome and non-COVID-19 acute respiratory distress syndrome[8].

To explore whether cytokine inhibitors could modulate the risk of infection, Simon et al.[3] undertook a study looking at SARS-CoV-2 seroprevalence among 793 patients with IMIDs in Bavaria, Germany, 534 of whom were receiving cytokine inhibitors and 359 of whom were not, as well as 971 healthy individuals and 285 health-care professionals. Overall, the prevalence of seroconversion was very low; only 46 people (2.2% of the total cohort) tested positive for anti-SARS-CoV-2 IgG antibodies. Compared with healthy individuals, the rate of positivity did not differ in patients with IMIDs not receiving cytokine inhibitors (relative risk (RR) 1.21; 95% CI 0.50–2.90) but was significantly lower among those who were receiving them (RR 0.32; 95% CI 0.11–0.99). Patients with IMIDs were less likely to have traveled or been in contact with an infected person than were the healthy individuals, but these behaviours were not related to use of cytokine inhibitors, suggesting the possibility that, in some way, these therapies might reduce susceptibility to COVID-19. A correlation between SARS-CoV-2 IgG antibody titre and severity of COVID-19 has also been reported[9], which could suggest that patients receiving cytokine inhibitors might have been exposed to SARS-CoV-2 infection but did not develop symptomatic illness. Unfortunately, the number of cases in the Simon et al.[3] study was too low to draw any more specific conclusions on the role of anti-cytokine therapies in COVID-19 disease severity, and therefore caution must be taken to not overinterpret these data, as there were in fact no significant differences (overlapping confidence intervals) in seroconversion rates between patients with IMIDs receiving and not receiving cytokine inhibitors.

Key advances

- Patients with rheumatoid arthritis, systemic lupus erythematosus or psoriasis, when analysed as a combined group, might have a slightly increased risk of death from COVID-19 compared to those without these diseases, although the role of disease activity and treatment in this risk estimation was not taken into account[2].
- Treatment with cytokine inhibitors could reduce the risk of SARS-SoV-2 infection (as measured by development of SARS-CoV-2 antibodies), although the mechanisms of this protective effect are not clear[3].
- Chronic use of glucocorticoids at moderate or high doses (≥10 mg per day prednisolone or equivalent) is associated with hospitalization for severe COVID-19[4].

Finally, the first publication from the COVID-19 Global Rheumatology Alliance (C19-GRA) examined factors associated with hospitalization among 600 cases of COVID-19 in patients with rheumatic diseases[4]. C19-GRA hosts an international database aiming to capture detailed data from rheumatology providers on COVID-19 outcomes in patients with rheumatic diseases, in order to address the knowledge gap regarding factors associated with severe disease. Since launching in March 2020, >5,000 cases globally have been reported via its European and global registries[10]. Of the 600 cases included in the analysis by Gianfrancesco et al.[4], 277 (46%) required hospitalization. As in the general population, older age and the presence of additional underlying health conditions were factors associated with hospitalization. Use of hydroxychloroquine was not associated with hospitalization (adjusted odds ratio (OR) 0.94; 95% CI 0.57–1.57) but use of high-dose glucocorticoids (≥10 mg per day of prednisolone-equivalent) was (adjusted OR 2.05; 95% CI 1.06–3.96). The study also included a preliminary analysis of DMARD exposure and found that compared with patients who were not receiving DMARDs, patients receiving biologic DMARDs (with TNF inhibitors being the most commonly prescribed) were less likely to be hospitalized (adjusted OR 0.46; 95% CI 0.22–0.93). Owing to the design of the C19-GRA database, it is not possible to conclude whether this observation is attributable to a higher than expected mortality rate in patients not receiving DMARDs or to a protective effect of biologic DMARDs. Patients receiving certain therapies, such as biologic DMARDs, might also be followed more closely in rheumatology clinics and, therefore, mild cases might be more likely to come to the attention of rheumatologists. Patients seen less frequently in rheumatology clinics might only come to the attention of the rheumatologist following hospitalization. Like the Williamson et al.[2] and Simon et al.[3] studies, interpretation of the findings of this study[4] in causal terms should be avoided.

Many questions about COVID-19 in patients with rheumatic diseases remain unanswered. Further studies are required to understand the differential risk between rheumatic diseases, the individual risk associated with use of the various classes of DMARDs, as well as the long-term effects of COVID-19 in this population, in order to advise on future social behaviour or treatment decisions. It is early days and as more cases (unfortunately) accrue, we will continue to learn more about this novel infection.

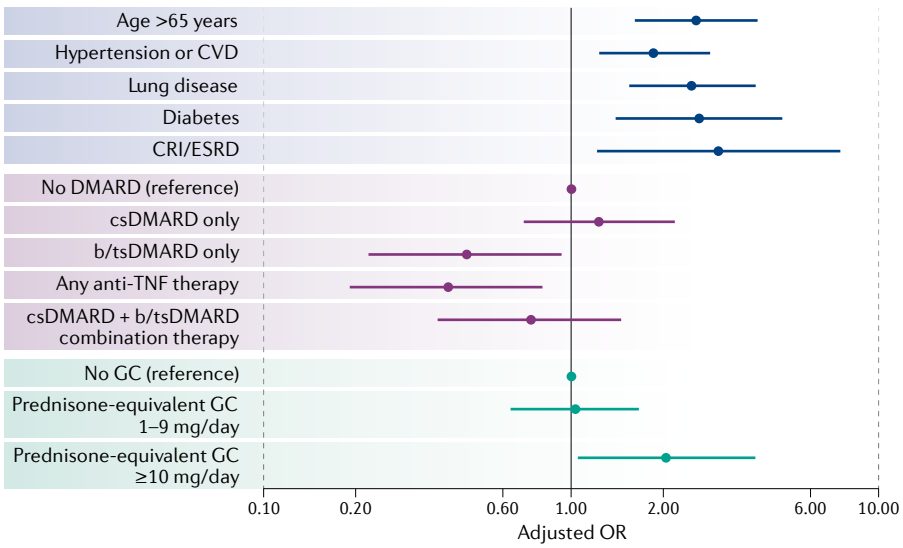

Fig. 1 | **Factors associated with hospitalization for COVID-19 infection.** This graph visualizes data from 600 patients with rheumatic diseases recorded in the COVID-19 Global Rheumatology Alliance international physician registry, reported by Gianfrancesco et al.[4]. Associations between the various factors and odds of hospitalization were estimated using multivariable-adjusted logistic regression and reported as odds ratios (ORs) with 95% confidence intervals. b/tsDMARD, biologic/targeted synthetic DMARD; CRI, chronic renal insufficiency; csDMARD, conventional synthetic DMARD; CVD, cardiovascular disease; ESRD, end-stage renal disease; GC, glucocorticoid.

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https://doi.org/10.1038/s41584-020-00562-2

1. World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard, https://covid19.who.int/ (2020).
2. Williamson, E. J. et al. Factors associated with COVID-19-related death using OpenSAFELY. *Nature* **584**, 430–436 (2020).
3. Simon, D. et al. Patients with immune-mediated inflammatory diseases receiving cytokine inhibitors have low prevalence of SARS-CoV-2 seroconversion. *Nat. Commun.* **11**, 3774 (2020).
4. Gianfrancesco, M. et al. Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann. Rheum. Dis.* **79**, 859–866 (2020).
5. Sepriano, A. et al. Safety of synthetic and biological DMARDs: a systematic literature review informing the 2019 update of the EULAR recommendations for the management of rheumatoid arthritis. *Ann. Rheum. Dis.* **79**, 760–770 (2020).
6. Siemieniuk, R. A. C. et al. Drug treatments for COVID-19: living systematic review and network meta-analysis. *BMJ* **370**, m2980 (2020).
7. Winthrop, K. L. & Mariette, X. To immunosuppress: whom, when and how? That is the question with COVID-19. *Ann. Rheum. Dis.* **79**, 1129–1131 (2020).
8. Leisman, D. E. et al. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. *Lancet Respir. Med.* https://doi.org/10.1016/S2213-2600(20)30404-5 (2020).
9. Zhang, B. et al. Immune phenotyping based on the neutrophil-to-lymphocyte ratio and IgG level predicts disease severity and outcome for patients with COVID-19. *Front. Mol. Biosci.* **7**, 157 (2020).
10. COVID-19 Global Rheumatology Alliance. Healthcare Provider Entered Registries, https://rheum-covid.org/provider-registry-gate/ (2020).

Acknowledgements
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Competing interests
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The views expressed are those of the authors and not necessarily those of the National Health Service, NIHR or the Department of Health.

RHEUMATOID ARTHRITIS IN 2020

Switching on resolution to treat RA moves closer to reality

Mauro Perretti

In inflammatory arthritides, such as rheumatoid arthritis (RA), synovial cells acquire aggressive and disruptive phenotypes that lead to joint disease. Three studies published in 2020 have described phenotypic variation in synovial cells, offering a novel perspective on the potential to resolve pathology and augment treatment options for patients with RA.

Chronic diseases, such as rheumatoid arthritis (RA), are characterized by progressive alterations in affected tissues and marked changes in the phenotype of resident stromal and blood-borne immune cells. These changes are considered terminal, hence current therapeutic options aim to stop further damage and local inflammation by blocking specific cytokines or other pro-inflammatory mediators. Owing to the complexity of RA, combinations of therapies are often used to hit multiple molecular and cellular targets. However, although effective in a proportion of patients, these therapies elicit a poor response in an equally relevant proportion of people. Moreover, even when a clinical response is obtained, remission is seldom achieved. In 2020, three studies have begun to define the cell phenotypes and subsets within diseased joints and have highlighted several cell subsets that are involved in the resolution of inflammation[1–3]. Such studies will provide guidance for the identification of therapeutic targets and steer the development of fresh therapeutic approaches that could be of value in overcoming unmet clinical needs in RA (FIG. 1).

The term 'resolution of inflammation' is used to identify a cluster of endogenous mediators and receptors that ensure an acute inflammatory response remains checked in space and time and eventually resolves[4]. When inflammation does not resolve and becomes chronic, physiological resolution processes might not be operative. From a therapeutic perspective, the reactivation of resolution-mediated responses in chronic diseases, through the engagement of specific pro-resolving receptors, offers the opportunity to switch cell behaviour to less aggressive phenotypes. Such a strategy would affect disease progression in a different way than therapies that inhibit specific pro-inflammatory pathways are able to[4].

To date, the potential effects of pro-resolving mediators and receptors have mostly been investigated in rodents and, although non-redundant regulatory effects on severity and duration of joint disease have been reported, little translation has occurred to human settings. In 2020, Alivernini et al. conducted a deep analysis of human synovial macrophages, applying an innovative comparative approach that included the use of synovial tissue from patients with RA in sustained remission[1]. This study capitalized on a 2019 study in which human synovial macrophages were described as clustering into distinct sub-populations[5]. By complementing single-cell analyses with other deep-phenotyping approaches, Alivernini and colleagues compared synovial macrophage profiles from healthy synovial tissue with those from patients with active RA and patients with RA in remission[1]. Such clear-cut clinical groupings underpin the importance of the scientific outcome of this study, which culminated in the identification of a pro-resolving MerTK$^+$CD206$^+$ synovial macrophage population. MerTK$^+$CD206$^+$ synovial macrophages were more abundant in healthy synovium and synovium from patients with RA in remission than in synovium from patients with active RA, in which this cell subtype was outnumbered by pro-inflammatory macrophages. Compared with other synovial macrophage subsets, MerTK$^+$CD206$^+$ cells displayed markers indicative of a reparative phenotype, including the scavenger receptor CD163 and the phosphatase DUSP1. Intriguingly, both CD163 and DUSP1 are upregulated by glucocorticoids, which are known to be beneficial in RA and to afford marked modulation of macrophage phenotypes in multiple disease settings[6].

Two other considerations are due. First, the localization of MerTK$^+$CD206$^+$ synovial macrophages to the lining layer of the RA synovium and the abundance of MerTK$^-$CD206$^+$ synovial macrophages in the sub-lining layer[1] confirm, at least in part, results from mouse models as to the architecture of pathogenic tissue in RA[7]. Second, once stimulated ex-vivo, MerTK$^+$CD206$^+$ synovial macrophages produce large amounts of the pro-resolving mediators resolvin D1 and IL-10, and small amounts of the pro-inflammatory cytokines IL-1 and IL-6 (REF.[1]), indicating that pro-resolving synovial macrophages have the potential to activate counter-regulatory circuits within a diseased joint. Notably, resolvin D1 is detected in synovial fluid from patients with RA and can accelerate the resolution of experimental arthritis[8]. Altogether, the results

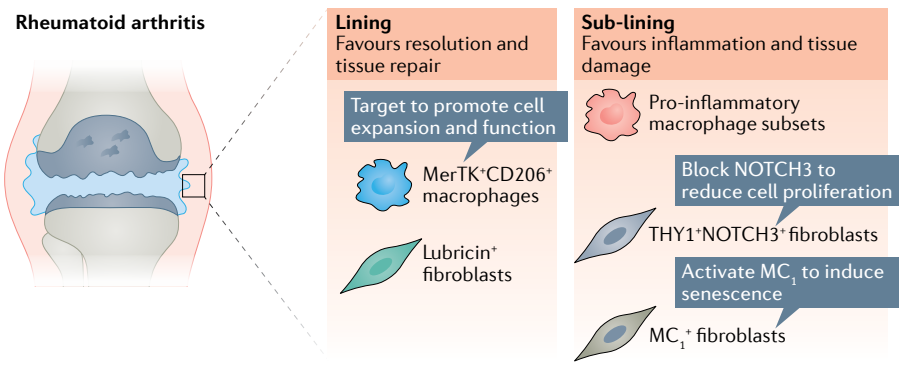

Fig. 1 | **Would being able to alter synovial cell phenotypes help to resolve RA?** A viable way to revert the course of joint disease lies in the possibility of switching the phenotype of synovial cells from pro-inflammatory to pro-resolving. Pro-resolving MerTK$^+$CD206$^+$ synovial macrophages exist in patients with rheumatoid arthritis (RA) in long-term remission, offering hope that these cells could potentially be harnessed for therapeutic purposes. Similarly, pro-inflammatory THY1$^+$ synovial fibroblasts invade the sub-lining tissue through a NOTCH3-mediated differentiation process, suggesting that strategies aimed at blocking NOTCH3 could be of therapeutic value. Meanwhile, a new approach to revert the fibroblast phenotype is provided by activation of a specific G protein-coupled receptor, the melanocortin type 1 receptor (MC_1), which leads to cellular senescence. MC_1-induced senescent fibroblasts acquire pro-reparative properties, which could favour remodelling to temper joint inflammation.

Key advances

- A subset of synovial macrophages with a pro-resolving phenotype can be used to predict remission in rheumatoid arthritis (RA)[1]; promoting the formation of pro-resolving macrophages might be therapeutically viable.
- Different fibroblast subtypes exist in RA synovium; blocking the NOTCH3 pathway can attenuate the proliferation of an aggressive subtype within the pannus in experimental arthritis with therapeutic benefit[2].
- Agonism of melanocortin type 1 receptor can temper the activation of synovial fibroblasts via the induction of senescence and is associated with a reparative phenotype[3].

of the study by Alivernini et al.[1] highlight the potential for resolution in the context of RA and provide links between pro-resolving macrophages and disease remission.

Advances in technology have also enabled the deep phenotyping and characterization of fibroblasts within the synovium. A groundbreaking study published in 2019 defined two main synovial fibroblast subsets, one in the lining and one in the sub-lining, that had distinct pathogenic properties[9], but did not identify the molecular signals responsible for fibroblast expansion within the synovium. In 2020, Wei et al. reported the existence of 'positional identity' in synovial fibroblast subsets in RA and characterized lubricin$^+$ lining synovial fibroblasts and THY1$^+$ sub-lining synovial fibroblasts, as well as an intermediate cell population[2]. In this study, the authors integrated single-cell transcriptomic data with confocal microscopy to reveal the existence of a cell-intrinsic transcriptional programme that functions alongside a gradient of gene expression patterns that span the synovium from perivascular regions to the lining layer. The so-called positional identity of the fibroblasts (the transcriptomic profiles for each subset; lining, sub-lining and intermediate) is rapidly lost when cells are cultured ex vivo; for example, the presence of endothelial cells is a prerequisite for keeping the transcriptomic profile of THY1$^+$ sub-lining fibroblasts in organoid cultures[2].

Wei and colleagues also identified NOTCH3, a receptor expressed on RA sub-lining fibroblasts, as important for determining fibroblast expansion within the synovium[2]. These sophisticated analyses of the human synovium were complemented by an assessment of the effect of NOTCH3 in experimental arthritis. The absence of NOTCH3 or the application of antibodies that block NOTCH3 signalling showed a remarkable inhibitory effect in the K/B×N serum transfer mouse model of arthritis, providing another parallel between human disease and experimental modelling of patho-pharmacology. The authors concluded that the discovery of a pathogenic sub-lining fibroblast population amplified in a NOTCH3-mediated manner could support the development of the fibroblast-directed therapies that are currently missing from those therapies available to rheumatologists[2].

In further support of a fibroblast-directed therapeutic approach, in 2020 Montero-Melendez et al.[3] reported that melanocortin receptor type 1 (MC_1) promotes senescence in proliferating synovial fibroblasts, yielding a resolving phenotype. Since the seminal work of Philip Hench, melanocortins such as adrenocorticotrophin have been known to be beneficial in RA, as well as in gout (reviewed elsewhere[10]). Over the past two decades, extra-adrenal receptors for melanocortins have been identified, a finding that explains the peripheral actions of these molecules. In this context, melanocortins have emerged as mediators endowed with anti-inflammatory properties and pro-resolving actions[10].

By studying the expression of several elements of the melanocortin system in RA synovial fibroblasts, Montero-Melendez et al. revealed the presence of MC_1 on these cells and discovered that selective activation of this receptor arrested cell proliferation[3]. This effect was associated with remarkable downstream consequences: MC_1-activated fibroblasts entered senescence, stopped releasing pro-inflammatory mediators and acquired a remodelling phenotype. Moreover, the NOTCH3 pathway was also inhibited by MC_1 activation, providing an interesting mechanistic link to the study by Wei et al.[2]. In mice with K/B×N serum transfer-induced arthritis, administration of a selective MC_1 agonist provoked fibroblast senescence in the pannus, which was functionally associated with an anti-arthritic effect[3]. Interestingly, cells with specific MC_1 receptor polymorphisms did not respond well to a pro-senescence treatment in vitro, indicating that pharmacogenomic analyses are required for the development of selective MC_1 agonists as novel anti-arthritic compounds. Potentially, such molecules would combine the known anti-inflammatory effects of melanocortins on immune cells[10] with the novel property of reverting the aggressive phenotype of synovial fibroblasts.

In summary, we are close to establishing whether our knowledge of mediators and mechanisms of the resolution of inflammation can affect the way RA is managed, both in terms of stratifying patients into clinically relevant subgroups and prediction of remission, and with respect to the development of innovative therapeutic strategies. The near future will provide the definitive answer on the validity of this approach.

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1. Alivernini, S. et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat. Med.* **26**, 1295–1306 (2020).
2. Wei, K. et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature* **582**, 259–264 (2020).
3. Montero-Melendez, T. et al. Therapeutic senescence via GPCR activation in synovial fibroblasts facilitates resolution of arthritis. *Nat. Commun.* **11**, 745 (2020).
4. Perretti, M. et al. Resolution pharmacology: opportunities for therapeutic innovation in inflammation. *Trends Pharmacol. Sci.* **36**, 737–755 (2015).
5. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* **20**, 928–942 (2019).
6. Toh, M. L. et al. Expression of mitogen-activated protein kinase phosphatase 1, a negative regulator of the mitogen-activated protein kinases, in rheumatoid arthritis: up-regulation by interleukin-1β and glucocorticoids. *Arthritis Rheum.* **50**, 3118–3128 (2004).
7. Culemann, S. et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature* **572**, 670–675 (2019).
8. Norling, L. V. et al. Proresolving and cartilage-protective actions of resolvin D1 in inflammatory arthritis. *JCI Insight* **1**, e85922 (2016).
9. Croft, A. P. et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* **570**, 246–251 (2019).
10. Montero-Melendez, T. ACTH: The forgotten therapy. *Semin. Immunol.* **27**, 216–226 (2015).

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PAEDIATRIC RHEUMATOLOGY IN 2020

MIS-C: early lessons from immune profiling

Lauren A. Henderson and Rae S. M. Yeung

Multisystem inflammatory syndrome in children (MIS-C) is a rare complication of SARS-CoV-2 infection that can result in serious illness in the paediatric population but our understanding of this syndrome is in its infancy. Translational studies in 2020 leveraging immune profiling have laid the foundation to enable further discovery in MIS-C.

In the early stages of the COVID-19 pandemic, healthy children were thought to have mild SARS-CoV-2 infections with favourable outcomes. In April 2020, reports began to emerge from COVID-19 epicenters describing clusters of children with features of Kawasaki disease and toxic shock syndrome[1,2]. This newly identified entity has many names and ultimately became known as multisystem inflammatory syndrome in children (MIS-C), as used by the WHO and CDC. As additional reports of MIS-C have surfaced, the clinical spectrum of this syndrome has broadened[3–5], and studies have begun to unveil its immune landscape, which could help in our understanding of this condition[6–10].

Emerging data show that MIS-C is characterized by the classic findings of inflammation, with fever as the cardinal feature, and multi-organ dysfunction that not only involves the skin, mucous membranes and heart but that also frequently affects the gastrointestinal, respiratory and neurologic systems (FIG. 1). However, the full clinical continuum of MIS-C is still being defined, and validated diagnostic criteria do not yet exist. As a result, researchers have employed varying case definitions of MIS-C so that patient populations are not necessarily comparable across studies[5]. This selection bias is important to consider because it affects our understanding of MIS-C.

MIS-C is temporally linked to SARS-CoV-2, and occurs as a late manifestation of or response to the infection, with cases peaking 3–6 weeks after the highest rate of SARS-CoV-2 infection (as measured by PCR positivity) in a given location[3,4]. The majority of patients had neutralizing antibodies to SARS-CoV-2, with greater titres of IgG antibodies than IgM antibodies, further indicating a preceding SARS-CoV-2 infection[2,3,6–10]. Building on these findings, Diorio et al.[8] evaluated the clinical and laboratory features of children with SARS-CoV-2 infections to clarify the differences between the early infectious phase of COVID-19 (severe COVID-19) and MIS-C. Compared with severe COVID-19, the PCR cycle thresholds for SARS-CoV-2 were higher for MIS-C, indicating a reduced viral burden and supporting the concept that MIS-C is a post-infectious process. Furthermore, this report identified demographics that differed between these two groups: patients with MIS-C were younger and less medically complex than patients with severe COVID-19. High levels of soluble C5b-9 (the membrane attack complex of the complement system) and evidence of microangiopathy on blood smears also suggested that endothelial dysfunction was central in the pathophysiology of both severe COVID-19 and MIS-C.

In a similar approach, Lee and colleagues evaluated the immunologic profile of MIS-C and identified the presence of T cell, B cell and natural killer cell cytopenias[7]. By comparing MIS-C to historic cohorts of Kawasaki disease (pre-pandemic Kawasaki disease), Lee et al. identified similarities and differences between these two childhood hyperinflammatory syndromes. Many patients with MIS-C had features of Kawasaki disease. However, the patients with MIS-C presented over a broader age range, had a greater degree of myocardial dysfunction, had more profound lymphopenia and thrombocytopenia, and more often showed signs of coagulopathy than the patients with pre-pandemic Kawasaki disease[2,7,10]. Whether MIS-C is distinct from Kawasaki disease or whether these two entities represent a continuum of the same clinical syndrome remains to be determined. Both reports by Diorio et al. and Lee et al. provide potentially useful diagnostic profiles of MIS-C; however, the results were derived from a small number of patients, and their generalizability awaits validation.

To gain further understanding of MIS-C, deeper immunophenotyping is required. Carter et al.[6] undertook this approach by studying 25 patients with MIS-C from the acute phase of illness through to convalescence using high dimensional cytokine and flow cytometry panels. At disease onset, treatment-naive patients with MIS-C had high serum levels of multiple cytokines, and the acute phase was associated with activated neutrophils and monocytes that expressed high levels of FcγRI. Circulating levels of $CD4^+$, $CD8^+$ and γδT cells were decreased early in the course of MIS-C compared with age-matched healthy individuals, with the exception of $CD4^+CCR7^+$ T cells. Although patients with MIS-C are able to generate neutralizing antibodies to SARS-CoV-2, the

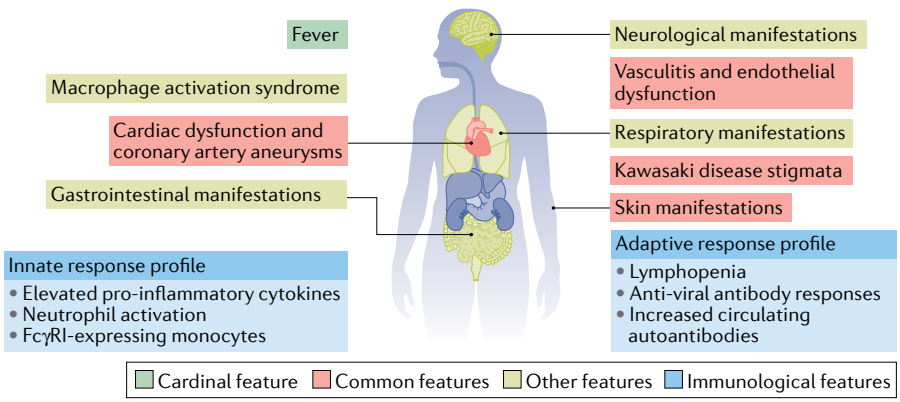

Fig. 1 | **Emerging clinical and immunological features of MIS-C.** Multiple organs are affected in multisystem inflammatory syndrome in children (MIS-C). Most patients have evidence of prior SARS-CoV-2 exposure, and Kawasaki disease features and cardiac dysfunction are common. The immune response in MIS-C is distinct from that during the acute SARS-CoV-2 infection, and is associated with elevated pro-inflammatory cytokines, activated neutrophils and monocytes, cytopenias (thrombopenia and lymphopenia) and appropriate anti-viral antibody responses detected to SARS-CoV-2.

Key advances

- The immune response in multisystem inflammatory syndrome in children (MIS-C) seems to be distinct from that during acute SARS-CoV-2 infection[8], but has both shared and distinct features compared with Kawasaki disease[7,10].
- The immune landscape shifts during the course of MIS-C, with the acute phase being characterized by activated innate immune cells and T cell and B cell lymphopenia, which normalize during recovery, and appropriate anti-viral antibody responses detected to SARS-CoV-2 (REF.[6]).
- MIS-C and Kawasaki disease might share plasma protein profiles but differ in autoantibody targets[9,10]; whether they are distinct or represent a continuum of the same clinical syndrome remains to be determined.

patients had lower levels of total B cells, effector B cells and class switched memory B cells in the blood than healthy individuals. After resolution of MIS-C, these observed innate and adaptive immune system changes normalized, and the frequency of plasmablasts and regulatory T cells increased. This work by Carter and colleagues identified a shifting immune landscape over the course of illness in MIS-C and highlighted several immune cell populations that might be important in either promoting disease or mediating recovery in MIS-C.

Multi-dimensional immune profiling was also employed in two other important publications from 2020 — Gruber et al.[9], and Consiglio et al.[10] — that evaluated immune responses in MIS-C compared with pre-pandemic Kawasaki disease and/or acute COVID-19. In principal component analysis (PCA) of circulating immune proteins, patients with MIS-C clustered separately from adults and children with acute COVID-19 (REFS[9,10]). Mass cytometry data from Gruber et al. showed a trend towards increased frequencies of circulating memory T cells in patients with acute COVID-19 compared with in patients with MIS-C, although most patients with MIS-C in this study were already being treated with immunomodulatory medications[9]. Comparisons of MIS-C with Kawasaki disease by Consiglio and colleagues yielded less conclusive findings. Patients with Kawasaki disease and patients with MIS-C clustered together in a PCA analysis of plasma proteins[10]. However, evaluation of immune cells by flow cytometry in MIS-C versus Kawasaki disease was limited owing to the small numbers of patients in the MIS-C group ($n = 3$).

Importantly, the work by Gruber et al.[9] and Consiglio et al.[10] has furthered our understanding of the humoral response in MIS-C. Both studies confirmed that patients with MIS-C generate appropriate antibody responses to SARS-CoV-2, as well as to other viruses. Compared with healthy individuals, patients with MIS-C had enrichment of both IgG and IgA autoantibodies directed towards peptides expressed in the endothelial, cardiac and gastrointestinal tissue as well as autoantibodies directed toward immune mediators[9]. Autoantibodies from both patients with Kawasaki disease and patients with MIS-C shared some targets, including proteins expressed by endothelial cells, whereas some autoantibodies were upregulated only in MIS-C or Kawasaki disease. Although these results are intriguing, the sample sizes were small, and it remains to be determined if these autoantibodies are primary mediators of disease in MIS-C or are generated secondarily as a result of tissue damage in the setting of infection.

Since MIS-C materialized as a complication of SARS-CoV-2 infections in children in early 2020, great strides have been made in characterizing the clinical presentation and immunophenotype of this syndrome, pointing to both innate and adaptive immunity together with vascular inflammation and endothelial dysfunction as important contributors to pathobiology. Yet, these studies represent only a beginning in our endeavour to understand MIS-C. To gain ground in this journey, future work will need to interrogate larger numbers of treatment-naive patients with MIS-C, along with appropriate febrile controls. To date, studies have focused on circulating immune perturbations; however, some cell populations of interest might have extravasated into affected tissues. Furthermore, the genetic susceptibilities that predispose patients to MIS-C are unknown, and the relationship between Kawasaki disease and MIS-C remains unresolved. The preliminary data generated by these translational research studies highlight the need for data sharing and cross-validation to bring disease understanding to a new level. Harmonizing case definitions and international collaborations will help accelerate the pace of advancement in MIS-C and make real change possible in the care and outcomes of this emerging condition.

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https://doi.org/10.1038/s41584-020-00566-y

1. Riphagen, S., Gomez, X., Gonzalez-Martinez, C., Wilkinson, N. & Theocharis, P. Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet* **395**, 1607–1608 (2020).
2. Verdoni, L. et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet* **395**, 1771–1778 (2020).
3. Dufort, E. M. et al. Multisystem inflammatory syndrome in children in New York State. *New Engl. J. Med.* **383**, 347–358 (2020).
4. Feldstein, L. R. et al. Multisystem inflammatory syndrome in U.S. children and adolescents. *New Engl. J. Med.* **383**, 334–346 (2020).
5. Abrams, J. Y. et al. Multisystem inflammatory syndrome in children associated with severe acute respiratory syndrome coronavirus 2: a systematic review. *J. Pediatr.* **226**, 45–54 (2020).
6. Carter, M. J. et al. Peripheral immunophenotypes in children with multisystem inflammatory syndrome associated with SARS-CoV-2 infection. *Nat. Med.* **26**, 1701–1707 (2020).
7. Lee, P. Y. et al. Distinct clinical and immunological features of SARS-COV-2-induced multisystem inflammatory syndrome in children. *J. Clin. Invest.* **130**, 5942–5950 (2020).
8. Diorio, C. et al. Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2. *J. Clin. Invest.* **130**, 5967–5975 (2020).
9. Gruber, C. N. et al. Mapping systemic inflammation and antibody responses in multisystem inflammatory syndrome in children (MIS-C). *Cell* **183**, 982–995 (2020).
10. Consiglio, C. R. et al. The immunology of multisystem inflammatory syndrome in children with COVID-19. *Cell* **183**, 968–981 (2020).

Competing interests
The authors declare no competing interests.

PSORIATIC ARTHRITIS IN 2020

New treatments for PsA meet targeted therapy goals

Philip J. Mease

Interest in therapies for psoriatic arthritis (PsA) has increased in response to recognition that many patients remain undiagnosed and are inadequately treated. In 2020, advances in PsA treatments have included phase III trials of an IL-23 inhibitor, head-to-head trials of IL-17 inhibition against TNF inhibition and updated EULAR treatment guidelines.

Interest in therapies for psoriatic arthritis (PsA) has expanded as more patients who were previously undiagnosed or misdiagnosed are recognized to have this condition, and switching between medications is becoming more commonplace in PsA as drugs lose efficacy over time. As such, there is an ongoing need for more effective and relatively safe medications to achieve treatment targets for PsA[1,2]. In 2020, several advances were made towards this goal, including publication of updated EULAR treatment guidelines for PsA[2], approval of a treatment with a new mechanism of action[3,4] and confirmation of the value of IL-17 inhibition in the PsA therapeutic armamentarium in head-to-head comparison trials[5,6].

PsA is a complex disease characterized by multiple 'domains' of disease activity, including arthritis, enthesitis, dactylitis, spondylitis, and skin and nail disease[1]. Disease in these domains impairs function and quality of life and causes tissue damage. Treatment should therefore be tailored to address each of these domains. Associated conditions and comorbidities can also influence therapy choice. Reflecting these considerations, in 2020, EULAR updated their recommendations for the management of PsA[2] from those published in 2015. Overarching principles were reiterated and included the importance of using a multidisciplinary approach that includes dermatologists; shared decision-making; the abrogation of inflammation to control symptoms, improve function and quality of life and prevent structural damage; and accounting for associated conditions and comorbidities. Treatment recommendations were updated to include recently approved medicines, such as Janus kinase inhibitors, and the contexts for their use in different disease domains (BOX 1). The new recommendations[2] have moved non-TNF inhibitor medication classes, such as the IL-17 and IL-12–IL-23 inhibitors, to an equal footing in the treatment of polyarthritis, particularly in patients with more skin disease. More consideration has also been given to non-articular domains of PsA, including enthesitis and axial disease, for which conventional synthetic DMARDs (csDMARDs) might not be efficacious, and biologic DMARDs should be used as first-line treatments.

IL-23 is an important cytokine in the pathogenesis of psoriasis, PsA and other related conditions. IL-23 is produced by dendritic cells and other immune cells and stimulates innate and adaptive immune cells to produce pro-inflammatory cytokines, which activate cells at tissue sites such as skin, synovium, entheses and bone, leading to inflammatory tissue destruction and bone remodeling[7]. In 2020, guselkumab, which inhibits the p19 subunit of IL-23, became the first of a new class of medications to be approved for the treatment of PsA. The approval was based on the results of two placebo-controlled phase III trials[3,4]: DISCOVER-1 and DISCOVER-2. Guselkumab had previously been approved for the treatment of psoriasis on the basis of a series of studies that demonstrated high degrees of efficacy and minimal safety signals[8].

DISCOVER-1 enrolled individuals with an inadequate response to or intolerance of csDMARDs, 30% of whom also had previously used at least one TNF inhibitor[3]. DISCOVER-2 enrolled individuals with an inadequate response to or intolerance of csDMARDs who had not been exposed to biologic DMARDs[4]. The dosing regimens for both studies were 100 mg guselkumab delivered subcutaneously every 8 weeks or every 4 weeks. In both studies, the primary outcome of an ACR20 response at week 24 was met, as were other important outcomes, including ACR50 and ACR70 responses, resolution of enthesitis and dactylitis, Minimal Disease Activity (MDA) targets and improvements in function, quality of life, fatigue and psoriasis. Inhibition of radiographic progression was statistically separated from placebo in those receiving guselkumab every 4 weeks in DISCOVER-2 ($P = 0.001$) but not in those receiving guselkumab every 8 weeks ($P = 0.07$), which is the currently approved dosing regimen[4]. The safety and tolerability profile of guselkumab in both studies was favourable, and there is no requirement for laboratory monitoring. Overall, it is valuable to add a new medication and class of therapy that has considerable efficacy and an excellent safety profile to the PsA therapeutic armamentarium.

In 2019, the 24-week results of a head-to-head trial of an IL-17 inhibitor (ixekizumab) against a TNF inhibitor (adalimumab) in PsA (SPIRIT H2H) were published[9]. These were followed in 2020 by the publication of a head-to-head trial comparing the IL-17 inhibitor secukinumab against adalimumab (EXCEED)[5] and the 52-week results of SPIRIT H2H[6]. In the double-blind EXCEED trial, 853 patients with PsA were enrolled and received either secukinumab or adalimumab; concomitant use of a csDMARD was not allowed[5]. The primary end point was an ACR20 response at 52 weeks, which was achieved by 67% of secukinumab-treated patients and 62% of adalimumab-treated patients (unadjusted $P = 0.0719$); thus, EXCEED failed to establish the superiority of secukinumab, which was the a priori goal of the study. Major non-articular domains improved, and an optimal target of treatment (MDA) was similarly achieved by both medications. More individuals achieved a 90% reduction in the Psoriasis Area and Severity Index (PASI) when receiving secukinumab than adalimumab, similar to the effect size in the SPIRIT H2H trial and confirming superiority of the IL-17 inhibition mechanism in psoriasis. In addition, the combined end point of simultaneous achievement of an ACR50 response and a 100% reduction in the PASI (PASI100) was achieved by 31% of those receiving secukinumab and 19% those receiving adalimumab (unadjusted $P = 0.0087$)[5], again similar to the effect size seen in the SPIRIT H2H trial.

Key advances

- Updated EULAR treatment recommendations for psoriatic arthritis (PsA) include newly approved medications, recognize the need for treatment tailored to the individual and encourage targets of remission or low disease activity[2].
- Guselkumab, the first of a new class of drugs that inhibit the p19 subunit of IL-23, was approved for PsA on the basis of results from two phase III clinical trials[3,4].
- Head-to-head trials of IL-17 inhibition against TNF inhibition confirmed similar efficacy of both therapies in musculoskeletal domains of PsA and superior efficacy of IL-17 inhibition in the psoriasis domain[5,6].

SPIRIT H2H demonstrated proportionally similar responses to EXCEED in musculoskeletal and skin outcomes (non-inferior in the former and superior in the latter) but met its primary end point of ACR50 and PASI100 responses at 24 weeks and demonstrated superiority of ixekizumab over adalimumab, as well as the secondary end points of non-inferiority in ACR50 response and superiority in PASI100 response[6,9]. Also different from EXCEED, SPIRIT H2H was an open-label, assessor-blinded study, which allowed flexibility in dosing depending on the degree of skin involvement each participant had. Furthermore, participants in SPIRIT H2H did not have to stop background csDMARDs, a flexibility that more closely mirrors clinical practice. A subanalysis of SPIRIT H2H looking at efficacy in the presence or absence of background methotrexate showed that for those receiving ixekizumab, results were similar regardless of whether methotrexate was being used, whereas for those receiving adalimumab, a lower percentage of patients not taking methotrexate achieved several end points, including the primary end point[6]. These findings have practical implications for the use of these agents as monotherapies, which some patients prefer so as to avoid adverse effects associated with methotrexate.

Although the EXCEED trial[5] failed to meet its primary end point whereas SPIRIT H2H[6,9] succeeded, in truth, this difference seems to be largely related to the choice and timing of the primary end point. When looking at the proportional outcomes of individual measures, and taking into account that one trial was double-blinded and the other open-label, the results are similar and provide a similar lesson. These trials[5,6,9] reveal that, in terms of speed of onset of action and magnitude of effect in musculoskeletal domains, IL-17 inhibitors are at least as effective as TNF inhibitors, which have been the mainstay of PsA treatment since the early 2000s, and that the effect of IL-17 inhibition on psoriasis and psoriatic nail disease is superior to TNF inhibitors. Although each medication class has its own specific safety issues, it seems that IL-17 inhibitors might also have a slightly better safety profile than TNF inhibitors[9]. Overall, we should now have confidence that IL-17 inhibitors can work as well as TNF inhibitors for most domains of PsA, and are actually better for some domains.

Box 1 | **Updated EULAR treatment recommendations for psoriatic arthritis**[2]

- Aim for remission or low disease activity, with regular assessment and therapy adjustment.
- NSAIDs can be used for musculoskeletal signs and symptoms.
- Consider local glucocorticoid injections as adjunct therapy and systemic glucocorticoids at the lowest effective dose.
- For polyarthritis, start conventional synthetic DMARDs (csDMARDs) rapidly; methotrexate is preferable depending on relevant skin involvement.
- For monoarthritis or oligoarthritis (particularly with poor prognostic factors such as structural damage, high erythrocyte sedimentation rate or C-reactive protein levels, dactylitis or nail involvement) consider a csDMARD.
- For peripheral arthritis in patients with an inadequate response to at least one csDMARD, start a biologic DMARD (bDMARD); an IL-17 inhibitor or IL-12–IL-23 inhibitor might be preferable depending on relevant skin involvement.
- For peripheral arthritis in patients with an inadequate response to csDMARDs and bDMARDs, or if bDMARDs are inappropriate, consider a Janus kinase (JAK) inhibitor.
- For mild disease in patients with an inadequate response to csDMARDs and for whom neither bDMARDs nor JAK inhibitors are appropriate, consider a phosphodiesterase 4 inhibitor.
- For unequivocal enthesitis in patients with an insufficient response to NSAIDs or local glucocorticoid injections, consider a bDMARD.
- For active, predominantly axial disease in patients with an insufficient response to NSAIDs, consider a bDMARD (typically a TNF inhibitor); an IL-17 inhibitor might be preferable depending on relevant skin involvement.
- For patients who fail to respond to or have intolerance to a bDMARD, consider switching to another bDMARD or a targeted synthetic DMARD, including one switch within a class.
- For patients who achieve sustained remission, consider cautious tapering of DMARDs.

To sum up, 2020 saw the publication of articles that updated the EULAR treatment recommendations for PsA[2], described the efficacy and safety of guselkumab[3,4], the first of a new class of medications for PsA, and further established the efficacy of IL-17 inhibitors in two head-to-head studies against adalimumab[5,6]. These studies advance the use of newer medications that address all, or virtually all, of the clinical domains of PsA, achieve sustainable treatment targets such as remission or low disease activity, and provide new treatment options for PsA.

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1. Ritchlin, C. T., Colbert, R. A. & Gladman, D. D. Psoriatic arthritis. *N. Engl. J. Med.* **376**, 957–970 (2017).
2. Gossec, L. et al. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. *Ann. Rheum. Dis.* **79**, 700–712 (2020).
3. Deodhar, A. et al. Guselkumab in patients with active psoriatic arthritis who were biologic-naive or had previously received TNFalpha inhibitor treatment (DISCOVER-1): a double-blind, randomised, placebo-controlled phase 3 trial. *Lancet* **395**, 1115–1125 (2020).
4. Mease, P. J. et al. Guselkumab in biologic-naive patients with active psoriatic arthritis (DISCOVER-2): a double-blind, randomised, placebo-controlled phase 3 trial. *Lancet* **395**, 1126–1136 (2020).
5. McInnes, I. B. et al. Secukinumab versus adalimumab for treatment of active psoriatic arthritis (EXCEED): a double-blind, parallel-group, randomised, active-controlled, phase 3b trial. *Lancet* **395**, 1496–1505 (2020).
6. Smolen, J. S. et al. Multicentre, randomised, open-label, parallel-group study evaluating the efficacy and safety of ixekizumab versus adalimumab in patients with psoriatic arthritis naive to biological disease-modifying antirheumatic drug: final results by week 52. *Ann. Rheum. Dis.* **79**, 1310–1319 (2020).
7. Nguyen, C. et al. Pathophysiology and inhibition of IL-23 signaling in psoriatic arthritis: A molecular insight. *Clin. Immunol.* **206**, 15–22 (2019).
8. Reich, K. et al. Maintenance of clinical response and consistent safety profile with up to 3 years of continuous treatment with guselkumab: Results from the VOYAGE 1 and VOYAGE 2 trials. *J. Am. Acad. Dermatol.* **82**, 936–945 (2020).
9. Mease, P. J. et al. A head-to-head comparison of the efficacy and safety of ixekizumab and adalimumab in biological-naive patients with active psoriatic arthritis: 24-week results of a randomised, open-label, blinded-assessor trial. *Ann. Rheum. Dis.* **79**, 123–131 (2020).

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CONNECTIVE TISSUE DISEASES IN 2020

New insights into the treatment of CTD-ILD

Athol U. Wells

Interstitial lung disease (ILD) can arise in a variety of connective tissue diseases (CTDs) and various treatment interventions are being explored. In 2020, advances in the treatment of CTD-associated ILD have included the re-evaluation of methotrexate-induced lung injury and emerging insights on anti-IL-6 therapy and anti-fibrotic therapy in this condition.

Interstitial lung disease (ILD) is a serious pulmonary complication of various connective tissue diseases (CTDs), including rheumatoid arthritis (RA) and systemic sclerosis (SSc), and is a major cause of morbidity and mortality. The past 12 months have seen major advances in our understanding of the treatment of connective tissue disease-associated ILD (CTD-ILD), including the reappraisal of methotrexate-induced lung toxicity and the emergence of novel therapies in this field[1–3].

The use of methotrexate in patients with RA-associated ILD (RA-ILD) has long been contentious. In its classical presentation, 'methotrexate lung' is an inflammatory pneumonitis. However, the concern that methotrexate might induce pulmonary fibrosis has led to the avoidance or discontinuation of methotrexate in patients with RA-ILD. The prevalence of methotrexate lung might have been greatly exaggerated by the perception that previously undiagnosed RA-ILD was due to methotrexate use. This assumption was challenged in 2017 by retrospective data suggesting that RA-ILD progression is slower with methotrexate therapy than with other agents[4], but the conclusions at the time were limited by the cohort size and the absence of a validation cohort.

In 2020, Juge and colleagues[1], together with other studies published in 2020 (REFS[5–7]), have re-evaluated the link between methotrexate use and RA-ILD (TABLE 1). In the study by Juge et al.[1], the populations comprised a French discovery cohort (patients with RA-ILD, $n = 100$; patients with RA but without ILD, $n = 165$) and a large multi-ethnic five-country validation cohort (RA-ILD, $n = 310$; patients with RA but without ILD, $n = 508$). In the combined cohort, the prevalence of usual interstitial pneumonia (UIP) or possible UIP (as assessed by CT) in patients with RA-ILD was 45.1%. In both cohorts, past methotrexate usage was strongly associated with a lower prevalence of RA-ILD, including after adjustments for age at RA onset, gender, ever-smoking, the duration of methotrexate exposure, the use of methotrexate at RA onset and the use of biologic agents. Methotrexate use was also associated with a longer time-interval to RA-ILD diagnosis (with a difference of 7.4 years in the combined cohort analysis). Importantly, historical differences in methotrexate usage in RA, classified by usual practice in defined time-periods, did not influence the findings.

These striking observations are consistent with less definitive studies, also published in 2020. Methotrexate usage in RA was not associated with the development or progression of lung disease in a small prospective cohort[5], and in retrospective analyses of two large databases[6,7]. Taken together, these data establish that chronic fibrotic lung disease is not an adverse effect of methotrexate therapy. Thus, historical estimates of the high prevalence of 'methotrexate lung' are exaggerated. Indeed, the delayed presentation of RA-ILD with methotrexate usage[1] suggests that methotrexate might eventually become a preferred treatment option in RA-ILD. However, such a change in practice requires future prospective evaluation and should not be based on a retrospective association without proof of causation.

In terms of treatment interventions in CTD-ILD, it remains unclear whether future treatments will be based on targeting single pathways in individual diseases (and perhaps in individual patients) or on a pleotropic approach that addresses a multiplicity of co-activated pro-fibrotic pathways common to many progressive fibrosing pulmonary disorders. Two notable studies from 2020 investigating the treatment of CTD-ILD stand at opposite ends of this spectrum: an analysis of data from the INBUILD trial[2] and the focuSSed trial[3].

The INBUILD study was a double-blind randomized placebo-controlled trial of nintedanib, a pleiotropic anti-fibrotic agent, in the treatment of progressive fibrosing ILDs other than idiopathic pulmonary fibrosis (IPF), and included a large subgroup of patients with CTD-ILD[8]. Trial enrolment required disease progression within the preceding 2 years despite management. The hypothesis was that in non-IPF disorders, patients with signs of IPF-like progression, despite real-world management, might have pro-fibrotic disease pathways similar to those in IPF. Nintedanib was strikingly efficacious, as judged by attenuation of forced vital capacity (FVC) decline in the whole study population, as well as across the two subgroups of those with UIP-like (and, thus, IPF-like) abnormalities (the majority of the participants), and those with non-UIP-like abnormalities on CT.

The aim of the 2020 study was to evaluate whether the whole-cohort treatment effects in the INBUILD study were common to the individual diseases grouped in the primary analysis[2]. The cohort was subdivided into five diagnostic subgroups (including a CTD-ILD subgroup, amalgamating RA-ILD,

Table 1 | **Studies in 2020 on methotrexate use and ILD presence and progression**

Study	Study design	Patients (patient number)	Primary findings
Juge et al.[1]	Retrospective: case-control with validation cohort	Patients with RA-ILD ($n = 410$) or with RA without ILD ($n = 673$)	Methorexate use was associated with a reduced prevalence and delayed onset of RA-ILD
Robles-Pérez et al.[5]	Prospective cohort	Patients with RA ($n = 40$)	Methotrexate use was not associated with the onset or progression of ILD
Ibfelt et al.[6]	Retrospective cohort (Danish national registry)	Patients with RA ($n = 30,512$)	Methotrexate use was not associated with an increased risk of ILD
Li et al.[7]	Retrospective cohort	Patients with RA without ILD at diagnosis ($n = 923$)	Methotrexate use was not associated with the onset or progression of ILD

ILD, interstitial lung disease; RA, rheumatoid arthritis.

Key advances

- Methotrexate use is probably not a notable cause of chronic fibrotic lung disease in rheumatoid arthritis (RA), and this drug might even delay the presentation of interstitial lung disease (ILD)[1].
- The efficacy of the anti-fibrotic agent nintedanib in the treatment of progressive fibrosing ILDs extends to the treatment of progressive connective tissue disease-associated ILD[2].
- Anti-IL-6 therapy is emerging as a potential new treatment option for systemic sclerosis-associated ILD[3].

SSc-ILD and ILD associated with other CTDs, amounting to 25.6% of the total INBUILD cohort). Multivariable analyses found no differences in treatment effects in any single diagnostic category in the whole cohort (diagnostic interactive P value = 0.41) and in subgroups with UIP-like and non-UIP-like abnormalities on CT.

Care is needed in interpreting these findings. Apparent minor differences in treatment effects between underpowered diagnostic subgroups are not meaningful, with inevitable and variable deviations from the overall cohort effect occurring because of chance. Furthermore, the exact amplitude of treatment effects in individual subgroups cannot be distilled from the analysis, given the wide confidence intervals associated with underpowered analysis. It can only be concluded that nintedanib will probably have an important future role in the management of patients with non-IPF ILD progression despite usual first-line therapies and that this group specifically includes patients with progressive CTD-ILD.

This important observation identifies a major new treatment opportunity in CTD-ILD, but clinical uncertainties must be acknowledged. The CTD-ILD subgroup was an amalgamation of individual CTDs, with RA-ILD predominating. Importantly though, the efficacy of nintedanib has also been shown in a stand-alone placebo-controlled study in SSc-associated ILD (SSc-ILD)[9]. Exact management prior to trial enrolment was not, and could not have been, standardized, because of treatment variations driven by variable systemic disease activity and treatment adverse effects in individual patients. Furthermore, the study does not establish the optimal timing of anti-fibrotic usage: specifically, the use of anti-fibrotic therapy as an initial treatment, possibly in combination with immunomodulation, in patients presenting with advanced fibrotic disease, has not been explored.

The dilemmas facing clinicians in the selection of therapies in CTD-ILD can only increase as novel agents are studied. With regard to SSc-ILD, the focuSSced study is a randomized controlled phase III trial of tocilizumab, an IL-6 receptor antagonist, in the treatment of patients with diffuse cutaneous SSc[3]. The results of this study are difficult to interpret with regard to routine SSc-ILD management for two reasons: the primary end point used and the study enrolment strategy. Active treatment was associated with attenuation of decline in FVC (as assessed by change in percentage of predicted FVC at 48 weeks); however, although FVC change was an important secondary end point, the primary end point (change in the modified Rodnan skin score at 48 weeks) was not met. Normally, a positive secondary end point, unsupported by the primary end point, would be viewed with scepticism. However, an earlier controlled phase II trial of tocilizumab in SSc observed exactly the same pattern of end point responsiveness: a change in the modified Rodnan skin score (the primary end point) was not observed but active treatment was associated in a reduction in the frequency of pulmonary function decline[10]. Sadly, these earlier observations did not inform the selection of the primary end point in the current study but the consistency of the FVC observations between the two studies is important.

Furthermore, defining the future role of tocilizumab in the treatment of SSc-ILD is complicated by the fact that the study did not selectively enrol patients with SSc-ILD, although the treatment effect was robust in the SSc-ILD subgroup. Mean pulmonary function tests at baseline were normal or only mildly reduced and, thus, the treatment group included a notable proportion of patients without clinically overt ILD. It is perhaps surprising, given this constraint, that a clear-cut overall FVC treatment effect was observed. Given the mildness of disease in the study population, it could be argued that a putative treatment benefit applies to limited SSc-ILD, perhaps when pro-inflammatory pathways are more prominent.

In summary, 2020 has seen advances highly relevant to future management algorithms in CTD-ILD. The new findings challenge the idea that methotrexate results in progressive ILD in RA and suggest that this drug might even have a future role in RA-ILD management, although this role remains to be established. Anti-IL-6 therapy can now be viewed as a treatment option in SSc-ILD, despite the limitations of the current studies; furthermore, the idea that this therapy might be more potent in the early stages of disease merits exploration. Anti-fibrotic therapies will probably have an important place in the management of CTD-ILD in future, but the optimal timing for their introduction and their use in combination with other treatments is, as yet, uncertain.

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1. Juge, P. A. et al. Methotrexate and rheumatoid arthritis associated interstitial lung disease. *Eur. Respir. J.* 2000337 (2020).
2. Wells, A. U. et al. Nintedanib in patients with progressive fibrosing interstitial lung diseases-subgroup analyses by interstitial lung disease diagnosis in the INBUILD trial: a randomised, double-blind, placebo-controlled, parallel-group trial. *Lancet Respir. Med.* **8**, 453–460 (2020).
3. Khanna, D. et al. Tocilizumab in systemic sclerosis: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir. Med.* **8**, 963–974 (2020).
4. Rojas-Serrano, J. et al. Rheumatoid arthritis-related interstitial lung disease (RA-ILD): methotrexate and the severity of lung disease are associated to prognosis. *Clin. Rheumatol.* **36**, 1493–1500 (2017).
5. Robles-Pérez, A. et al. A prospective study of lung disease in a cohort of early rheumatoid arthritis patients. *Sci. Rep.* **10**, 15640 (2020).
6. Ibfelt, E. H. et al. Methotrexate and risk of interstitial lung disease and respiratory failure in rheumatoid arthritis: a nationwide population-based study. *Rheumatology (Oxford)* **11**, keaa327 (2020).
7. Li, L. et al. A retrospective study on the predictive implications of clinical characteristics and therapeutic management in patients with rheumatoid arthritis-associated interstitial lung disease. *Clin. Rheumatol.* **39**, 1457–1470 (2020).
8. Flaherty, K. R. et al. Nintedanib in progressive fibrosing interstitial lung diseases. *N. Engl. J. Med.* **381**, 1718–1727 (2019).
9. Distler, O. et al. Nintedanib for systemic sclerosis-associated interstitial lung disease. *N. Engl. J. Med.* **380**, 2518–2528 (2019).
10. Khanna, D. et al. Safety and efficacy of subcutaneous tocilizumab in systemic sclerosis: results from the open-label period of a phase II randomised controlled trial (faSScinate). *Ann. Rheum. Dis.* **77**, 212–220 (2018).

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Immunogenicity of biologic agents in rheumatology

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Abstract | Biologic agents have become a core component of therapeutic strategies for many inflammatory rheumatic diseases. However, perhaps reflecting the specificity and generally high affinity of biologic agents, these therapeutics have been used by rheumatologists with less consideration of their pharmacokinetics than that of conventional synthetic DMARDs. Immunogenicity was recognized as a potential limitation to the use of biologic agents at an early stage in their development, although regulatory guidance was relatively limited and assays to measure immunogenicity were less sophisticated than today. The advent of biosimilars has sparked a renewed interest in immunogenicity that has resulted in the development of increasingly sensitive assays, an enhanced appreciation of the pharmacokinetic consequences of immunogenicity and the development of comprehensive and specific guidance from regulatory authorities. As a result, rheumatologists have a greatly improved understanding of the field in general, including the factors responsible for immunogenicity, its potential clinical consequences and the implications for everyday treatment. In some specialties, immunogenicity testing is becoming a part of routine clinical management, but definitive evidence of its cost-effectiveness in rheumatology is awaited.

Phage display
A technique whereby an antibody-variable sequence is displayed on the outside of a bacteriophage that contains the DNA encoding the variable sequence, enabling the screening and selection of bacteriophages containing the genetic sequence of interest.

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Biologic agents now form part of the therapeutic armamentarium for most inflammatory rheumatic diseases. Since their early development, an emerging feature of biologic agents has been their propensity to provoke an immune response against themselves (known as immunogenicity), most notably, the generation of anti-drug antibodies (ADAs), which can have clinical consequences; for example, in the early 1990s, researchers noted that repeat courses of OKT3 (a mouse monoclonal antibody that recognizes CD3) had limited clinical efficacy because the mouse antibodies were highly immunogenic in humans. The humanization of monoclonal antibodies (and the subsequent development of 'fully human' monoclonal antibodies produced in transgenic mice carrying human immunoglobulin genes, or by phage display or single-cell cloning) have subsequently reduced the immunogenicity of biologic agents[1]. In parallel with the development of biosimilar biologic agents[2,3], ways of measuring immunogenicity have become more sophisticated and assays more sensitive over the past 10–15 years[4,5], which has led to a better understanding of immunogenicity and its consequences and a deeper knowledge of the pharmacokinetics of biologic agents[6,7]. Hence, many of the factors that provoke immunogenicity and the formation of ADAs are now well understood, although others still remain unclear.

The consequences of immunogenicity can vary and are influenced by the nature of the ADAs (for example, the antibody isotype) and the consequent immune complexes that form with the biologic agent. Although current strategies for designing monoclonal antibodies are aimed at minimizing immunogenicity via progressive humanization and innovative quality-by-design risk-minimization manufacturing methods, it still cannot be abolished completely. Thus, researchers have developed strategies to predict and lessen ADA formation. Another development has been the publication of algorithms for monitoring serum drug concentrations and ADAs in clinical practice, although the cost-effectiveness of such testing in rheumatology has not been robustly demonstrated.

In view of the growth in knowledge in the field that has occurred over the past few years, it is timely to comprehensively review the available data. In this Review, we summarize what is known about biologic agent pharmacokinetics and the factors that influence immunogenicity, including knowledge gleaned from agents used for non-rheumatic indications. We discuss the potential consequences of immunogenicity and the methods available for measuring ADAs and serum drug concentrations. We also summarize data related to the biologic agents that have been licensed for rheumatic indications, including data from studies on treatment switching,

Key points

- All biologic agents are immunogenic and many pathways influence their bioavailability, including patient-specific factors, disease-specific features and genetic background.
- The potential consequences of immunogenicity range from no clinical consequences to reduced therapeutic efficacy, infusion reactions and, rarely, serum sickness or anaphylaxis.
- Group level pharmacokinetic models have consistently shown that anti-drug antibodies (ADAs) result in decreased serum drug concentrations and reduced efficacy.
- The most important difference between available immunogenicity assays is the degree to which the assay is drug tolerant.
- Coadministration of anti-proliferative and/or immunosuppressive agents such as methotrexate decreases ADA formation and maintains serum drug concentrations via various mechanisms.
- Regular monitoring of serum drug and ADA levels has been proposed but not yet instigated into rheumatological practice, mainly owing to a lack of cost-effectiveness data.

and discuss implications for clinical practice, including the pros and cons of therapeutic drug monitoring.

Pharmacokinetics and immunogenicity

All biologic agents are immunogenic and many pathways influence their bioavailability, immunogenicity being just one of them (FIG. 1). The pharmacokinetics of monoclonal antibodies are influenced by proteolytic catabolism, target-binding capability and specific receptor-determined clearance mechanisms, including Fcγ receptor-mediated immunoglobulin clearance. IgG antibodies, including monoclonal antibody-based biologic agents, are recycled and salvaged by the neonatal Fc receptor (FcRn; also known as Brambell receptor) on vascular endothelial and reticuloendothelial system cells (such as monocytes, macrophages and dendritic cells)[8]. The structure of the monoclonal antibody itself, including its amino acid sequence, allotype, route of administration, dosing regimen and duration of treatment, can also influence both the pharmacokinetics and immunogenicity[9,10]. Another important immunogenic factor is the presence of aggregates in the therapeutic protein preparations, although modern production processes are designed to eliminate this source of immunogenicity[11].

Patient-specific factors such as a low serum albumin concentration, high BMI and/or drug target levels can also affect the clearance of biologic drugs. Concomitant administration of immunosuppressive and anti-proliferative agents such as methotrexate, azathioprine, mycophenolate mofetil and leflunomide decrease ADA formation[4] and might additionally raise biologic agent concentrations in blood[12]. Disease-specific features also affect immunogenicity. In general, lower amounts of ADAs have been reported in patients with spondyloarthritis (SpA) than in those with rheumatoid arthritis (RA) in longitudinal studies and randomized controlled trials (RCTs), despite background anti-proliferative agents being less commonly used in patients with SpA[13–15]; furthermore, the incidence of ADAs is higher in patients with more active disease. Studies have also linked variability in HLA type, HLA alleles and ethnicity to immunogenicity[16,17].

Interestingly, certain TNF inhibitors (such as infliximab, adalimumab and etanercept) seem to stabilize TNF trimers, resulting in up to 50-fold higher circulating concentrations of TNF, which plateau and stabilize during a course of treatment[18,19]. Because TNF is in a complex with its inhibitor, it is inactive, and these apparently high concentrations do not reflect disease activity. However, as early as 4 weeks into treatment, patients who later develop ADAs have lower TNF concentrations than those who do not, perhaps reflecting clearance of TNF–TNF inhibitor complexes by low affinity ADAs[18]. Furthermore, even very low concentrations of a circulating biologic agent (such as <0.1 μg/ml of adalimumab) can quantitatively neutralize TNF, suggesting a pharmacodynamic effect that might extend for many months after treatment is discontinued[18].

Early work in mouse models identified the cell-binding capacity of a monoclonal antibody therapy as a predictor of immunogenicity[20]; although it was possible to induce tolerance to antibodies that recognize soluble targets, it was difficult to tolerize to antibodies that bind to cell surface antigens[21]. The mechanisms linking the cell-binding capacity of monoclonal antibodies to immunogenicity might reflect antibody-induced cell lysis and/or enhanced presentation of immunogenic epitopes, particularly the antibody idiotype[22,23]. Although the mechanism has not been defined, following cell lysis and uptake by a phagocyte, the idiotype of the antibody might be protected from proteolysis by being bound to antigen, thereby increasing the likelihood of the presentation of immunogenic epitopes derived from the idiotype. Allotypic differences in human IgG1 antibodies might also contribute to or potentiate immunogenicity[24], although data from T cell assays and MHC-associated peptide proteomics assessing the immunogenicity of tocilizumab suggest that allotypic differences in human IgG1 are not a notable risk factor for the induction of immunogenicity with this agent[25].

In theory, humanized antibodies should be less immunogenic than chimeric antibodies owing to the presence of less non-human protein sequences in the variable region that might be recognized as foreign. Absolute evidence to support this theory is lacking as no head-to-head comparisons of an equivalent chimeric and humanized monoclonal antibody have been performed. The best evidence comes from an indirect comparison of chimeric and humanized anti-CD52 (Campath) monoclonal antibodies[26]: 15 out of 17 transplant recipients who received Campath-1G (a chimeric rat monoclonal antibody) developed ADAs, whereas none of the 12 transplant recipients who received Campath-1H (a humanized monoclonal antibody) developed ADAs. Furthermore, infliximab (the only chimeric monoclonal antibody among the five available TNF inhibitors) is more immunogenic than any of the other four TNF inhibitors[4] (TABLE 1). Indeed, an infusion reaction is more likely to occur with infliximab than with golimumab (a humanized monoclonal antibody)[27]. However, whether a humanized monoclonal antibody is more immunogenic than a fully human monoclonal antibody is unknown. Even with fully human antibodies, complementarity-determining regions (CDRs; also referred to as hypervariable regions) are still immunogenic, owing to the high variability of

Single-cell cloning
A technique whereby the antibody-encoding genetic material is extracted from a human B cell clone that produces the antibody of interest.

Idiotype
The collection of sequences (idiotopes) that form the antigen-binding site of an antibody.

Humanized antibodies
Antibodies in which the complementarity determining regions of a human antibody have been replaced with those from a mouse antibody of interest to create an antibody with the specificity of the mouse antibody in the context of a mostly human sequence.

Chimeric antibodies
Antibodies in which the variable region of a mouse antibody of interest has been genetically fused with a human constant region to create an antibody that retains the specificity of the mouse antibody in the context of a human constant region.

Fully human antibodies
Antibodies that contain only sequences derived from human genes.

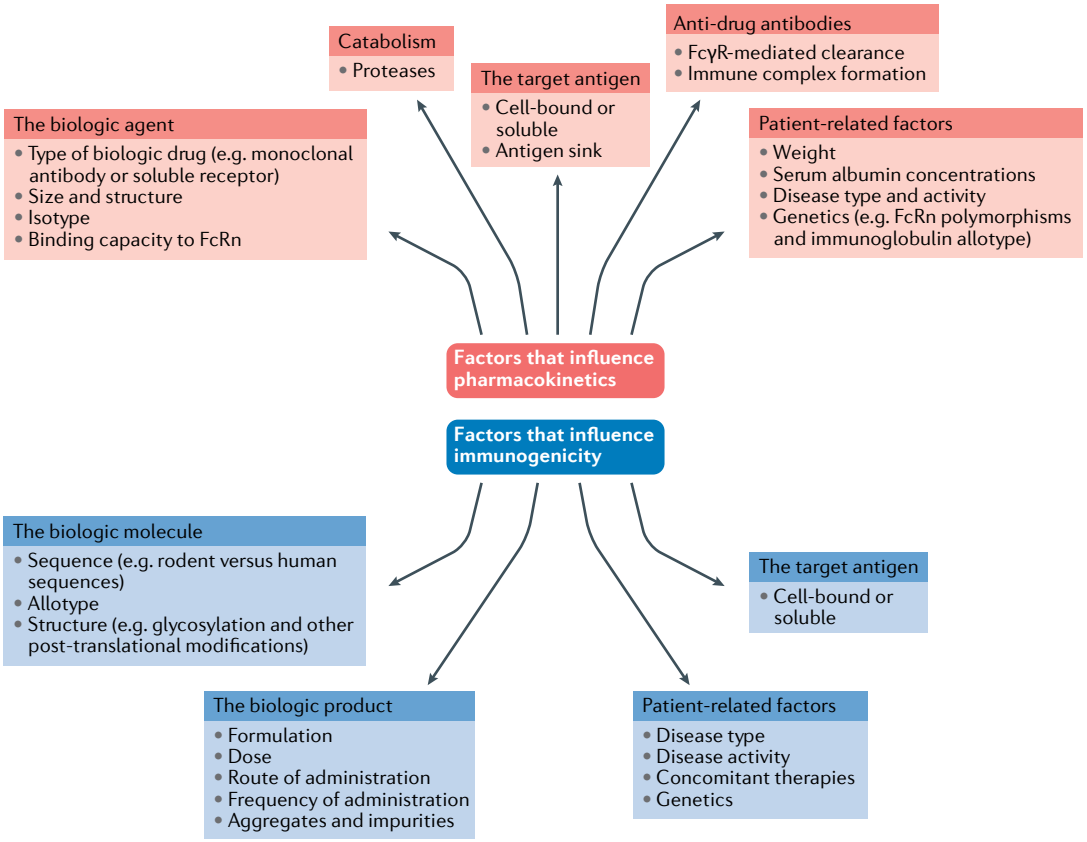


Fig. 1 | **Factors that influence the pharmacokinetics and immunogenicity of biologic agents.** Various factors can influence the pharmacokinetics of a biologic agent, including factors relating to the drug itself (the type of biologic agent, the size and structure, the isotype or the binding affinity for neonatal Fc receptor (FcRn)), the target antigen (whether the antigen is cell-bound or soluble and its level of expression), the presence of proteases that can digest the drug, the development of anti-drug antibodies (including the formation of immune complexes and accelerated clearance via Fcγ receptor (FcγR) binding) and patient-related factors (the disease being treated and disease activity, the weight of the patient, serum albumin concentrations and genetic factors). A number of factors can also influence the immunogenicity of a biologic agent, including factors relating to the drug itself (the primary sequence, the allotype and post-translational modifications such as glycosylation), the target antigen (soluble or cell-bound), the final drug product (formulation, dosing regime and route of administration or the presence of impurities or aggregates) and patient-related factors (the disease being treated and disease activity, concomitant therapies such as methotrexate and genetic factors).

CDRs following recombination events and somatic hypermutation occurring throughout life and a consequent lack of central tolerance. Whether murine CDRs are more immunogenic than human CDRs has not been formally assessed in a head-to-head comparison of a humanized and fully human monoclonal antibody. However, fully human antibodies manufactured using homologous recombination (such as golimumab, ustekinumab, secukinumab and sarilumab) are associated with lower incidences of ADAs than humanized antibodies (TABLE 1). The debate around the immunogenicity of chimeric, humanized and fully human antibodies has continued for a long time; however, the data reviewed in this article indicate that fully human antibodies are potentially the least immunogenic, an opinion shared by developers and regulatory agencies.

Pegylation and enzyme replacement

Information relevant to immunogenicity can be extrapolated from the experience of enzyme replacement therapy for haemophilia A and haemophilia B, which are caused by genetic deficiency of coagulation factors. In factor VIII and IX replacement therapy for haemophilia, the amount of cross-reactive immunological material produced by the patient determines the degree of immunogenicity and the success of the treatment[28]. Most patients with haemophilia A make some amount of factor VIII, even if the protein is non-functional, and anaphylaxis is rare, whereas the majority of patients with haemophilia B have large deletions or a minor deletion with a stop codon in the gene encoding this protein, and anaphylaxis is common[28]. Similarly, the tolerability of enzyme replacement therapy, in terms of the rate of adverse reactions such as arthralgias, injection site reactions and serum sickness, is associated with the amount of endogenous protein present, whether mutated and/or non-functional, rather than with its bioactivity[29]. Thus, the treatment of Gaucher disease (which is caused by a hereditary deficiency of the enzyme glucocerebrosidase) with recombinant glucocerebrosidases is frequently successful, owing to residual endogenous production of the enzyme. By contrast, in Pompe disease

Cross-reactive immunological material
An endogenous protein in the recipient that is immunologically similar to the replacement therapy.

(which is caused by deficiency of the lysosomal enzyme acid α-glucosidase), treatment with alglucosidase alfa (an analogue of α-glucosidase) can be complicated by nephrotic syndrome resulting from renal deposition of antigen–ADA complexes[30,31]. In mouse models of enzyme deficiencies, the use of anti-proliferative agents has been most effective if they are administered along with the first dose of enzyme replacement therapy[32].

Pegvaliase, a pegylated derivative of the enzyme phenylalanine ammonia lyase (which metabolizes phenylalanine), is approved for the treatment of phenylketonuria. One analysis of the long-term safety of pegvaliase treatment assessed the immunogenicity of pegvaliase during induction, upward titration and maintenance dosing regimens in 261 adults with phenylketonuria[33]. All patients developed ADAs to the phenylalanine ammonia lyase part of pegvaliase, the titres of which peaked at 6 months and stabilized thereafter; most patients also developed transient ADAs to the polyethylene glycol (PEG) component of pegvaliase, which peaked at 3 months and returned to baseline by 9 months. The binding of ADAs to pegvaliase led to the formation of circulating immune complexes, complement activation and hypersensitivity reactions, which most frequently occurred during early treatment and were associated with injection site reactions and arthralgias or arthritis but not with abnormalities in renal function or other serious adverse events, and was consistent with circulating immune complex-mediated type III hypersensitivity reactions. As the pegvaliase dosage increased, blood phenylalanine concentrations decreased over time, as did the amount of circulating immune complexes and complement activation. Overall, these data suggest that patients can develop tolerance to the PEG component of pegvaliase with continued regular administration, although ADAs to phenylalanine ammonia lyase can also persist.

Pegylation has also been utilized to prolong the serum half-life of biologic products with applications in rheumatology, including certolizumab pegol (a humanized $F(ab')_2$ fragment TNF inhibitor) and two pegylated uricases (pegloticase and pegadricase) used to treat patients with tophaceous gout. As uricase has not been retained in humans owing to a missense and frameshift mutation during evolution, it is very immunogenic without retained cross-reactive immunological material; the PEG conjugated to uricase is also immunogenic, perhaps reflecting broad exposure to PEGs in food additives, skin creams and personal lubricants.

In two phase III RCTs assessing the efficacy of intravenous administration of pegloticase for the treatment of chronic gout, 42% of patients were identified as 'complete responders' (maintaining serum uric acid concentrations below 6 mg/dl for more than 80% of the time)

Table 1 | **Frequency of anti-drug antibody formation in rheumatic diseases**

Biologic agent or biosimilar	RA	PsA	JIA	AS	Psoriasis	Range	Refs
Abatacept	2–20% (7)	ND	2–11% (2)	ND	ND	2–20% (9)	4
Adalimumab	0–51% (33)	0–54% (8)	6–33% (6)	8–39% (9)	0–51% (12)	0–54% (84)	4
Adalimumab biosimilar (5)[a]	31.8–43.2% (4)	ND	ND	ND	36.8–55.2% (2)	31.8–55.2% (6)	6
Certolizumab pegol	2.8–37% (7)	ND	ND	ND	21% (1)	3–37% (14)	4
Etanercept	0–13% (25)	0% (3)	0–6 % (2)	0 (4)	2–5% (5)	0–13% (37)	4
Etanercept biosimilars (2)[a]	0.3% (1)	ND	ND	ND	0% (1)	0–0.3% (2)	6
Golimumab	2–10% (11)	6% (1)	ND	0–6.4% (2)	ND	0–19% (22)	4
Infliximab[b]	8–62% (48)	15–33% (3)	26–42% (2)	6.1–69% (10)	0–41% (12)	0–83% (114)	4
Infliximab biosimilars (3)[a,b]	48.2–53.0% (3)	ND	ND	25.0% (1)	ND	22.9–53.0% (6)	6
Ixekizumab	ND	5.2–10.3% (2) with methotrexate; 8.6–12.0% (2) as monotherapy	ND	ND	ND	5.2–12.0% (2)	111
Rituximab[b]	0–21% (8)	ND	ND	ND	ND	0–21% (8)	4
Rituximab biosimilars (3)[a,b]	10.0–17.6% (5)	ND	ND	ND	ND	10.0–17.6% (5)	6
Secukinumab	ND	0–0.35% (6)	ND	0–0.69% (6)	0–1% (8)	0–1% (14)	4,108
Tocilizumab	0–16% (14)	ND	1–8% (3)	ND	ND	0–16% (17)	4
Ustekinumab	ND	8–11% (3)	ND	ND	4–8.6% (10)	1–11% (15)	4

The numbers in this table refer to percentages of patients with anti-drug antibodies across various randomized controlled trials, with the number of trials in parentheses. Adapted from REF.[4], Springer Nature Limited. AS, ankylosing spondylitis; JIA, juvenile idiopathic arthritis; ND, no data; PsA, psoriatic arthritis; RA, rheumatoid arthritis. [a]Refers to the number of biosimilars for a particular biologic agent. [b]All patients in these trials were receiving background methotrexate therapy.

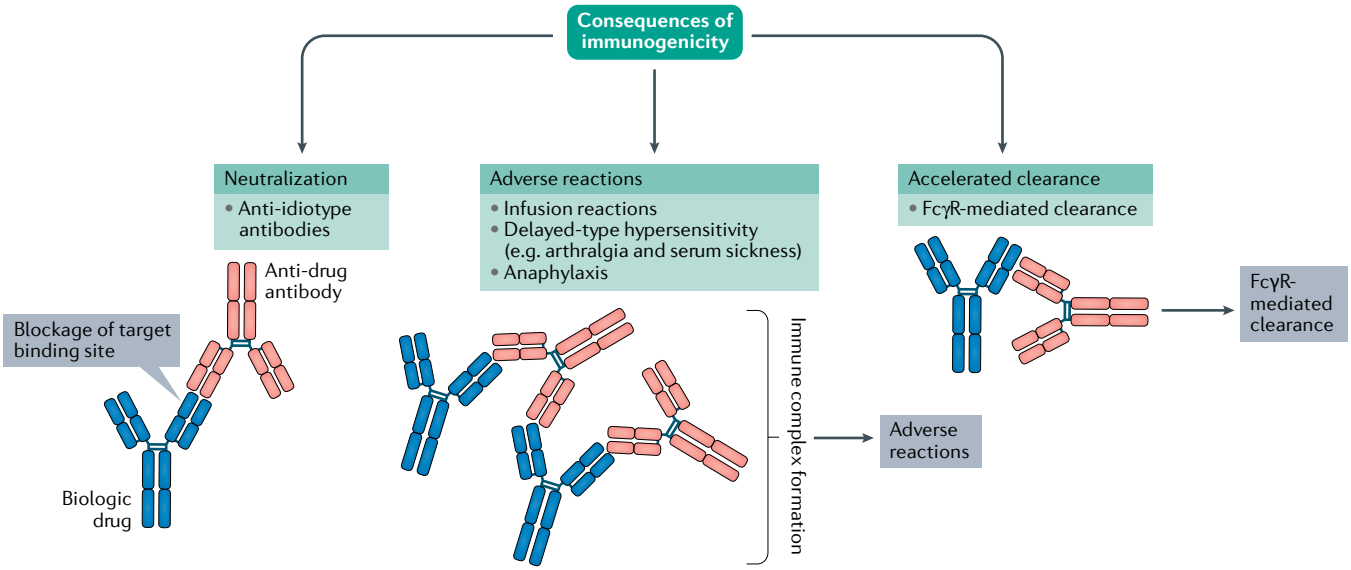


Fig. 2 | **Consequences of immunogenicity.** Anti-drug antibodies can be neutralizing if they bind at, or close to, the antigen-binding site of the therapeutic biologic agent (such as occurs with anti-idiotype antibodies). Anti-drug antibodies can also accelerate clearance of the drug via immune complex formation and binding to Fcγ receptors (FcγRs) and can lead to a variety of adverse reactions (depending on the size of the immune complex), including infusion reactions, delayed-type hypersensitivity reactions and, rarely, anaphylaxis.

at months 3 and 6 compared with 0% of those who received placebo ($P < 0.001$)[34]. Complete response was associated with complete resolution of at least one target tophus in 45% of patients who received treatment every 2 weeks[35]. Infusion reactions occurred in 26% (22 out of 85) of the patients in the treatment group, including anaphylaxis in four individuals, compared with only 5% (2 out of 43) of the patients in the placebo group; infusion reactions were predicted by serum uric acid concentrations >6.0 mg/dl before the infusion[36]. The infusion reactions were associated with the presence of ADAs, resulting in the monitoring of serum uric acid levels before each infusion being recommended in the product label which, in a post hoc analysis, would have reduced the incidence of infusion reactions to 2%[37].

High-titre ADAs to pegloticase in the phase III RCTs were associated with a loss of treatment response owing to reductions in serum drug concentrations (which thus caused serum uric acid concentrations to increase); the ADAs were not neutralizing and primarily recognized the PEG moiety, leading to accelerated drug clearance[38]. In a phase II RCT of pegloticase in 30 patients with refractory symptomatic gout, 7 of whom were organ transplant recipients, 5 of the patients had a durable response to therapy and only 1 patient developed ADAs[39]. All the patients had been receiving anti-proliferative and/or immunosuppressive drugs, including cyclosporine and tacrolimus. Subsequent case series and clinical trials of pegloticase have reported successful persistent therapeutic outcomes and even 'recapture' of lost clinical effect following initiation of anti-proliferative background therapy using methotrexate, azathioprine, mycophenolate mofetil or leflunomide[40–45].

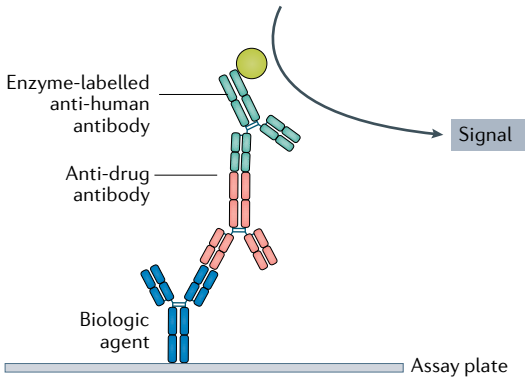
To try to mitigate immunogenic responses, the other pegylated uricase, pegadricase, is co-administered with a proprietary biodegradable nanoparticle, ImmTOR. ImmTOR encapsulates the immunomodulator rapamycin (sirolimus) to mitigate the formation of ADAs, ostensibly by delivering a tolerogenic message to dendritic cells as they are exposed to pegadricase in the spleen and liver[46]. An RCT comparing pegadricase with pegloticase is currently underway[47].

Consequences of immunogenicity

The potential consequences of immunogenicity on the pharmacokinetics and pharmacodynamics of biologic agents vary (FIG. 2). For the majority of patients, immunogenicity to biologic agents, particularly to fully human monoclonal antibody therapies, has no clinical consequences. In some individuals, ADAs are associated with reduced therapeutic efficacy, either because of immune complex formation and accelerated drug clearance and/or because of neutralizing antibodies that block monoclonal antibody binding to the epitope binding site[48]. At least some immune complexes are removed by the reticuloendothelial system in the spleen and liver[49]. ADA formation is also linked to certain adverse events following biologic agent therapy, such as injection site reactions and/or infusion reactions, the latter being more common with infliximab; less common adverse reactions to biologic agent therapy include serum sickness and anaphylaxis (which occurs rarely).

At a group level, the presence of ADAs is typically associated with lower drug concentrations and reduced efficacy and/or a secondary loss of response; but at the level of the individual, a high degree of variability exists[12]. Characteristics of the ADAs are also important. Low-affinity ADAs, which are typical in individuals with pre-existing reactivity to the drug (such as can occur with pre-existing reactivity to PEG), seem unlikely to interfere with therapy, although this theory has not been

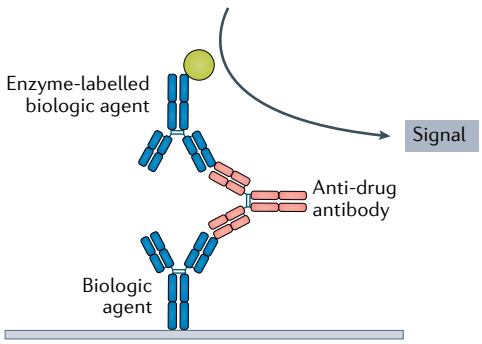
a **Indirect ELISA**



Strengths
- Easy to use
- High throughput
- Inexpensive
- Generic reagents and instrument

Weaknesses
- High background
- High risk of false-positive results
- Misses low-affinity antibodies
- Biologic agent immobilization can mask epitopes
- Requires species-specific secondary reagent
- Not suitable for monoclonal antibody products

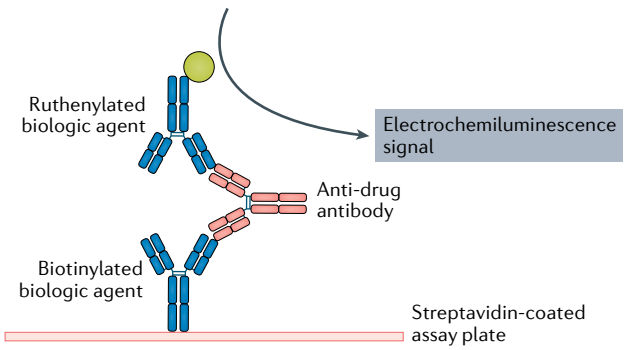
b **Bridging ELISA**



Strengths
- Easy to use
- High throughput
- Inexpensive
- More specific and selective than indirect ELISA
- Suitable for monoclonal antibody products
- Generic reagents and instrument

Weaknesses
- Affected by the presence of the biologic drug and other serum components (for example, anti-human Ig molecules)
- Misses low-affinity antibodies
- Might not detect IgG4 and IgM antibodies
- Antigen labelling might alter the antigen

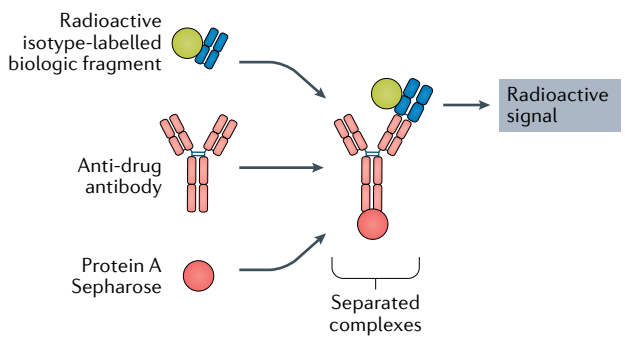
c **Electrochemiluminescence assay**



Strengths
- High throughput
- Large dynamic range
- Minimally affected by matrix
- High tolerance to therapeutic
- Suitable for monoclonal antibody products

Weaknesses
- Affected by the presence of the biologic drug and other serum components (for example, anti-human Ig molecules)
- Misses low-affinity antibodies
- Might not detect IgG4 antibodies
- Antigen labelling might alter the antigen
- Needs specific equipment and reagents

d **Radioimmunoassay**



Strengths
- Moderate throughput
- High sensitivity
- Can be specific
- Inexpensive

Weaknesses
- Requires radiolabelled antigen
- Requires special equipment and safety precautions
- Decay of radiolabel might affect the antigen stability
- Can be isotype-specific

Fig. 3 | **Immunogenicity screening assays.** The figure shows commonly used anti-drug antibody (ADA) detection immunoassay formats and their strengths and weaknesses. **a** | For indirect enzyme-linked immunosorbent assays (ELISAs), the biologic agent is coated on the assay plate, which captures any ADAs present in the sample; these antibodies are then detected by an anti-human IgG antibody conjugated to an enzyme that provides a colorimetric or chemiluminescent signal. **b** | Bridging ELISAs involve coating the biologic agent directly onto an assay plate. Following an optional acid-dissociation pretreatment step, the patient sample is added and any ADAs present are captured by the plate-bound drug. The captured ADAs are then detected using an enzyme-labelled biologic agent, so that any ADAs present must bind to two biologic agents (a plate-bound and a labelled biologic agent) to emit a signal. Other ELISA methods designed to measure ADAs make use of anti-human λ-chain-conjugated antibodies as the detector antibody instead of a labelled biologic agent. **c** | In an electrochemiluminescence immunoassay, following an acid-dissociation pretreatment step, the sample is incubated with ruthenylated and biotinylated forms of the biologic agent, which bind to any ADAs that are present. The sample is then added to a streptavidin-coated plate, which captures the ADA–biologic agent complexes. In the presence of tripropylamine and on application of an electric current, the ruthenium produces a chemiluminescent signal. **d** | In radioimmunoassays, protein A Sepharose captures the serum ADAs, which bind to radiolabelled fragments of the biologic agent, and the radioactivity of the separated complexes are measured. An important benefit of this method is that the biologic agent is in solution and has a low probability of denaturing as a result of coating. Moreover, the risk of false positives owing to binding of rheumatoid factor or non-specific antibody binding is low. Disadvantages of the radioimmunoassay method include the complexity of the test, the long incubation time and safety concerns around the use of radioactive material.

systematically studied. By contrast, repeated therapy in an individual already sensitized to the biologic agent might lead to ADAs of increasingly high affinity[50]. The amplification of pre-existing endogenous antibodies[51] presents a new challenge in the assessment of immunogenicity and its clinical relevance. In the future, it will be helpful to identify the conditions that allow ADA formation to remain limited to a low-titre, transient, IgM response with few clinical effects or that promote seroconversion.

Immunogenicity can be categorized by the functional effect of the ADAs on serum drug concentrations, that is, whether the ADAs are binding (non-neutralizing) antibodies that do not affect drug–target interactions, or neutralizing antibodies that bind to the pharmacologically active site of the biologic agent, thereby physically interfering with the ability of the drug to bind to its target[52]. The clinical importance of testing for binding ADAs or neutralizing ADAs in patients being treated with a monoclonal antibody therapy is not clear. Although neutralizing ADAs might have a direct negative effect on functional drug concentrations, the major safety concern for this type of ADA relates to enzyme replacement therapies, for which cross-reactivity to the endogenous counterpart can lead to life-threatening adverse effects[32]. However, no specific safety concerns have been reported for neutralizing ADAs to monoclonal antibody therapeutics. Nonetheless, binding ADAs might indirectly decrease drug concentrations by increasing drug clearance via immune complex formation.

As highlighted in the next section, harmonization of immunogenicity assessments is necessary. Specifically, harmonization of the type of assessments and assay strategies used for measuring the immunogenicity of a biologic product, including measurement of the immunogenicity of different biologic products in the same therapeutic class, as well as implementation of similar study and laboratory protocols to obtain comparable data, would improve our understanding of the clinical consequences of immunogenicity.

Monitoring therapeutics and ADAs

Knowledge of immunogenicity and methodologies to evaluate unwanted immune reactions have advanced considerably since the introduction of biologic therapies. The precision and sensitivity of immunogenicity assays have improved over time and will continue to do so. Consequently, the use of these new assays has highlighted a higher rate of immunogenicity than previously thought[53,54]. Clinicians should be knowledgeable about these developments and how differences between assay types might influence interpretation of the assay test results. Although the assessment of immunogenicity was of great importance during drug development, the arrival of biosimilars and the requirement to compare these drugs with their reference products in RCTs has generated new clinical information on the immunogenicity of already approved biologic therapies in rheumatology[52,55]. During this time, the technology used in immunoassay platforms has evolved, meaning that the assay platforms originally used to monitor reference products during development might now be outdated.

Information relevant for the assessment of the effects of immunogenicity on overall clinical benefit-to-risk ratios for therapeutic proteins is complex and distributed across many different sections of the regulatory dossier. Moreover, essential background information on the intrinsic immunogenic potential of the molecule, and how extrinsic factors (such as the product quality, patient variables and dose regimen) might interact to influence the clinical manifestations, is often missing. For this reason, a draft guideline on immunogenicity assessment from the EMA and guidance from the FDA on immunogenicity testing formally recommend that an "integrated summary of immunogenicity" be included in the product's regulatory dossier[56,57].

ADA testing

The detection and assessment of ADAs is complex, and results can be influenced by the assay utilized. Hence, it is important to utilize specific and approved strategies when evaluating immunological responses. Screening tests must be sensitive, specific and able to recognize all isotypes of ADAs to a given biologic agent. Platforms for assessing immunogenicity include different types of immunoassay, such as enzyme-linked immunosorbent assays (ELISAs), electrochemiluminescence immunoassays (ECLIAs) and radioimmunoassays, as well as different immunoassay formats, such as direct, indirect, bridging and competitive formats[53,54,58] (FIG. 3).

ELISAs and ECLIAs are the major platforms of choice for ADA detection because such immunoassays offer high sensitivity and throughput. Regardless of the clinical relevance of low-affinity or high-affinity ADAs, an assay should be capable of detecting a reasonable range of ADA affinities. With indirect ELISAs, ADAs are captured by the biologic agent immobilized on a plate (FIG. 3a). A major disadvantage of such assays in the setting of humanized and fully human therapeutics is high background caused by the enzyme-labelled anti-human

antibody cross-reacting with the plate-bound capture antibody. Additionally, fixation of the biologic agent to the solid surface during plate coating can alter its conformation and which epitopes are exposed, decreasing the sensitivity of the assay and leading to the potential for cross-reactivity[59]. These drawbacks have been circumvented by bridging ELISAs, in which the non-labelled biologic agent is directly immobilized on the plate in the correct orientation to allow bridging of the ADAs to the labelled biologic agent[60,61] (FIG. 3b). Disadvantages include the occurrence of false positives because of non-specific binding and loss of low-affinity ADAs during repeated washes. Sandwich versions of ELISAs are also available and are more selective and specific than either indirect or bridging formats; however, they still might lose low-affinity ADAs during washing steps[62]. Continuing improvements of immunoassays have resulted in ECLIAs, which utilize the same principles as an ELISA but use a ruthenium-conjugated protein rather than an antibody for detection, and are therefore more sensitive for detecting monoclonal antibodies[63] (FIG. 3c). Radioimmunoassays are based on high-sensitivity assay techniques to measure concentrations of antigens by the use of antibodies, or alternatively to detect antibodies that recognize a specific antigen. These assays measure the presence of an antigen with very high sensitivity. In a radioimmunoassay, the target antigen is labelled radioactively and bound to its specific antibodies. Serum is added to initiate a competitive reaction between the labelled antigens from the preparation and the unlabelled antigens from the serum for the specific antibodies (FIG. 3d). The competition for the antibodies releases a certain amount of labelled antigen, which is proportional to the ratio of labelled to unlabelled antigen. However, comparison of data in the literature seems to show that ECLIA is more sensitive than radioimmunoassay and is less affected by drug interference, with the advantage that patient and study heterogeneity is not a limiting factor for study comparisons.

The most important distinction between immunogenicity assays is the extent to which the assays are drug-tolerant; in other words, how sensitive an assay is to the presence of the biologic agent in the serum, which, when present in equivalent concentrations to ADAs, causes the formation of immune complexes[64]. The concentration of the biologic agent in the sample needed to interfere with ADA detection depends on the amount of ADA present in the patient sample, meaning that the drug tolerance of an assay will be higher for serum with high ADA titres and lower for serum with low ADA titres. To detect ADAs with high confidence, assays must have high specificity and sensitivity. Moreover, it is important to minimize drug interference in an assay, which can be achieved by several strategies, such as sample pretreatment, the use of drug-tolerant assays and the use of competing antibodies. For example, in bridging ELISAs, ADAs link non-labelled biologic agent to labelled biologic agent; thus, immune complex formation precludes recognition of the bridging moiety and can lead to underestimation of immunogenicity. To overcome this technical weakness, drug-tolerant assays have been developed by adding an acidic or basic pretreatment step designed to dissociate ADA–drug complexes in serum samples[65]. Other technical advances that have been used to increase the drug tolerance of assays include affinity capture elution and the use of nanoparticles or magnetic beads. Data from such assays consistently show that low-affinity ADAs are detectable at 2–4 weeks after the initial biologic agent dosing and that the majority of ADAs are evident within 12–24 weeks[66].

Whether drug-tolerant assays are more useful than other assays in clinical practice is a subject of debate. These assays detect ADAs that decrease drug serum concentrations in the patient, but also detect low-affinity antibodies that do not cause clinically relevant changes in the pharmacokinetics of the drug. Furthermore, large ADA–drug complexes are eliminated rapidly from the circulation, which can lead to immunogenicity being underestimated[62]. By contrast, drug-sensitive assays typically only reveal ADAs when serum trough concentrations are below clinically relevant concentrations. Therefore, clinical judgements made on the basis of drug-tolerant assay results must be carefully assessed, given that the strong associations between immunogenicity and clinical effects were mostly established using drug-sensitive assays[67–69].

Irrespective of the technique used to detect ADAs, assay validation parameters should include cut-off points, sensitivity, drug tolerance, specificity, precision, dilution range of the serum and reproducibility[62,70]. In the absence of reference standards, these assays are simply quasi-quantitative. As a consequence, to correctly interpret ADA test results, the dose, timing of administration and serum drug concentrations should be determined concomitantly with immunogenicity. In practical pharmacokinetic terms, the assessment of clinical immunogenicity requires collecting samples at the end of the drug elimination phase (that is, when the drug is at its lowest concentration) immediately before the next administration, to avoid drug interference in the assay. Repeated testing is useful for determining whether the ADAs are transient. If necessary, a positive test result should be confirmed by incorporating an excess of biologic agent into the assay, which will reduce the signal of a truly positive ADA result. The detection of ADAs is typically followed by assessments of the magnitude (titre) of the ADA response, especially in late-stage clinical studies. ADA titres provide more useful information for the interpretation of ADA data and for determining relationships with clinical outcomes than mass concentrations. Hence, ADA titres are usually determined by running positive samples in serial dilution and reporting the titre as the reciprocal of the last dilution at which the sample scores are negative. Samples verified as ADA-positive might also be subsequently tested for the presence of neutralizing ADAs using cell-based bioassays or competitive ligand-binding assays. Cell-based bioassays, which monitor the function of the biologic agent in the presence of neutralizing ADAs, are recommended by the FDA. However, cell-based approaches can be laborious and difficult to develop, despite the provision of validation guidelines provided by the FDA and EMA[59,60].

Table 2 | **Characteristics of TNF inhibitors**

Characteristic	Adalimumab	Certolizumab pegol	Etanercept	Golimumab	Infliximab
Molecular structure	Fully human IgG1κ monoclonal antibody	Pegylated F(ab´)^2 fragment of humanized IgG1κ monoclonal antibody	Fusion protein of a human TNFR2 and IgG1 Fc region	Fully human monoclonal antibody	Chimeric (mouse and human) monoclonal IgG1κ antibody
Binding specificity	TNF	TNF	TNF and lymphotoxin	TNF	TNF
Anti-nuclear antibody induction	+++	+	+	++	++
FcγR binding	++	–	+/–	ND	++
Transmembrane TNF neutralization	+++	+++	++	+++	+++
Reverse signalling (apoptosis)	+++	–	+/–	+++	+++
Reverse signalling (cytokine suppression)	+++	+++	++	+++	+++
Antibody-dependent cytotoxicity[a]	+++	–	+/–	+++	+++
Complement-dependent cytotoxicity[a]	+++	–	+/–	+++	+++
Associated with lupus-like syndrome	Yes	Yes	Yes	Yes	Yes
Associated with demyelination or neuropathies	Yes	Yes	Yes	Yes	Yes

Adapted with permission from REF.[128], Elsevier. FcγR, Fcγ receptor; ND, no data; TNFR2, TNF receptor 2. [a]Examined under in vitro conditions.

Therapeutic drug monitoring

Population pharmacokinetic models have consistently shown that ADAs that recognize TNF inhibitors can increase the clearance rate of the drug, resulting in decreased serum drug concentrations, as occurs with infliximab, adalimumab, golimumab and certolizumab pegol[71,72]. Therefore, the pharmacokinetics of the therapeutic protein can also be used as a marker of immunogenicity. Therapeutic drug monitoring requires a different methodology to immunogenicity assays but similarly lacks a single standard technique or algorithm. Differences between pharmacokinetics data are not caused by a lack of correlation between results obtained using different methodologies, as clinical decisions are often similar regardless of the assay used. However, 20–30% of therapeutic drug monitoring results are potentially incorrect because of differences in how the cut-off levels of the assays are determined[73]. Therefore, more evidence from RCTs is needed during the development of biologic agents to identify and optimize the use of immunogenicity assays in clinical practice. To improve therapeutic decision-making, the same assays and cut-off values should be used during the follow-up of each individual patient.

In terms of implementing therapeutic drug monitoring and immunogenicity testing in clinical practice, usually >20 samples a day are required to enable the laboratory to make results clinically available in a cost-effective matter. As a result, the clinician frequently only has the results of the last test just in time for the next scheduled infusion. To surmount this disadvantage, rapid point-of-care tests for measuring serum concentrations of TNF inhibitors are becoming increasingly available[73]. Quantitative and qualitative validation of these tests against conventional ELISAs has been successful[72]. Such rapid testing offers many advantages, such as enabling testing during outpatient visits for patients who do not respond to therapy and who need to be monitored by a nurse, physician or researcher before their next infusion. Because of the rapidity of obtaining the test results (results are typically obtained within 15–20 min), treatment can be adjusted immediately rather than at the subsequent infusion (which typically occurs 6–8 weeks later)[63].

Immunogenicity of biologic therapies

In this section, we review published immunogenicity data for biologic agents that are licensed or approved for use for inflammatory arthritis (including RA, psoriatic arthritis (PsA) and ankylosing spondylitis).

TNF inhibitors

Infliximab. Being chimeric, infliximab is the most immunogenic of the TNF inhibitors[4] (TABLE 2). The presence of ADAs to infliximab is generally associated with reduced serum infliximab concentrations, decreased pharmacodynamic effects and clinical responses and a greater risk of infusion reactions[4]. ADAs are formed to the mouse portion of the monoclonal antibody, which includes the epitope binding region, and are generally of the IgG, IgA or IgM isotype or, less commonly, the IgE isotype[74].

The formation of large, irregularly shaped ADA immune complexes occurs in patients with high serum concentrations of both infliximab and ADAs, as can happen

during and following intravenous administration[49]. Following infusions, peak serum concentrations of infliximab can reach as high as 100–150 mg/ml, approximately ~1% of the total serum IgG antibodies; the presence of equimolecular concentrations of the drug and ADAs can lead to the rapid formation of soluble IgG complexes[49]. Individual anti-infliximab antibody clones have different propensities to form dimers, tetramers, hexamers and even larger complexes in vitro[49]. Large immune complexes are rapidly internalized by macrophages and preferentially cleared in vivo, leaving only dimer complexes in the circulation. Large disorganized complexes, especially those larger than hexamers, activate the complement cascade and result in subsequent infusion reactions, which therefore represent a type III hypersensitivity reaction (immune-complex mediated) rather than a type I IgE hypersensitivity reaction, which is consistent with the rarity of detection of IgE ADAs[28,49].

ADA formation is lower in patients with RA receiving higher doses of infliximab than in patients receiving lower doses; induction regimens and background therapy with methotrexate and/or leflunomide also reduce the incidence of ADAs in patients with RA[60]. ADAs to infliximab or its biosimilar CT-P13 occur at a lower rate in those with SpA than in those with RA, even in the absence of background therapy[13,15]. ADA titres also increase with the duration of therapy.

As related in an earlier section, some ADA responses are conventional, T cell-dependent, immune reactions[22]. Researchers have identified various immunogenic T cell epitopes in the variable chain regions of infliximab and rituximab by deriving $CD4^+$ T cell lines generated from 15 healthy individuals[75]. Six of the nine T cell epitopes identified could stimulate peripheral blood mononuclear cells from patients sensitized against infliximab or rituximab, promoting the secretion of a diverse range of cytokines. Thus, the identification of neo-epitopes and their MHC binding capabilities might, in some cases, predict the immunogenicity of therapeutic monoclonal antibodies. Removing such epitopes from the amino acid sequence of the therapeutic monoclonal antibody could decrease its immunogenicity; however, this approach would require an entirely new clinical development programme as the modified biologic would be considered a new monoclonal antibody therapy rather than a biosimilar.

Immunogenicity data are also available for three of the infliximab biosimilars[6] (TABLE 1). On the basis of more sensitive assays, the incidence of ADAs in patients with RA receiving background methotrexate approximates 50%, the majority of which are neutralizing. Positivity for ADAs is associated with lower serum drug concentrations, reduced clinical responses and infusion reactions. Epitope recognition was similar between biosimilars and reference product, showing a similar antigenic presentation. Potentially immunogenic epitopes are mainly present in the variable light chain and heavy chain but are also present in the Fc domain.

Adalimumab. Adalimumab is a fully humanized anti-TNF antibody that was developed using phage display substitution, a method that was the subject of the Nobel prize for chemistry in 2018 (REFS[76,77]). Even with humanization, heavy and light variable chain amino acid sequences adjacent to the epitope binding site within the CDR of the monoclonal antibody are broadly immunogenic in healthy volunteers as well as in patients with autoimmune diseases. This immunogenicity has been confirmed by prominent $CD4^+$ T cell responses to adalimumab in samples from around 100 healthy individuals[78].

ADAs to adalimumab are predominantly neutralizing ADAs of the IgG1 or IgG4 isotype that circulate as small dimeric immune complexes. These ADAs have been extensively studied, particularly in Dutch cohorts of patients with RA or PsA[74,79–85]. The majority of patients develop ADAs within the first 28 weeks of treatment; high titres are associated with low or undetectable serum drug concentrations, reduced clinical responses and, less commonly, injection site reactions. ADA levels increase with longer duration of therapy. Induction regimens (in patients with Crohn's disease) and the use of background therapy with methotrexate or other anti-proliferative agents maintain adalimumab blood concentrations and decrease ADA formation[18,86,87]. Coadministration of methotrexate prolongs the half-life of adalimumab by 40–50%[88]; an effect that is dose-dependent[89], distinct from its effects on immunogenicity, and presumably caused by inhibition of Fc-mediated clearance mechanisms[66] or increased FcRn expression in tissues[90,91]. Methotrexate does not have a similar effect on prolonging the half-life of the TNF inhibitors etanercept or certolizumab pegol. Methotrexate also reduces serum TNF concentrations and, owing to reduced target-mediated drug disposition, contributes to increased TNF inhibitor concentrations and improved clinical responses[91]. However, given the high quantity of TNF inhibitor compared with TNF, a reduction in target-mediated drug disposition does seem to be a plausible explanation. Another hypothesis is that methotrexate suppresses early B cell and T cell responses towards the biologic agent, leading to immune modulation that is dependent, in part, on red blood cell methotrexate polyglutamate concentrations and thus the dose and duration of methotrexate administration[92,93]. To date, the CONCERTO trial[89] is probably the RCT that has best addressed the appropriate dosing of methotrexate with adalimumab, although the results were confounded as all patients had been receiving methotrexate before enrolment. Given the long half-life of red blood cell methotrexate polyglutamate concentrations, the effects of changes in drug doses reported in the trial might have been delayed.

Serum concentrations of 5–8 μg/l adalimumab are associated with optimal clinical benefit in patients with RA or PsA, although a threshold serum adalimumab concentration and a predictor of remission could not be identified in patients with Crohn's disease[6]. Immunogenicity data are also available for the six adalimumab biosimilars[3] (TABLE 1). ADAs to the biosimilars are consistently detected in approximately 40–50% of patients with RA receiving background methotrexate and in 50–60% of patients with psoriasis receiving biosimilar monotherapy. The majority of these ADAs

Target-mediated drug disposition
When the binding of a drug to its target affects the pharmacokinetics of the drug.

(50–100%) are neutralizing ADAs, although results can vary depending on the type of assay utilized.

Golimumab. Golimumab is a fully human anti-TNF monoclonal antibody that was produced using homologous recombination in genetically modified mice. Overall, the incidence of ADAs to this biologic agent is low, typically ranging from 2% to 19%[4]. Nonetheless, as with adalimumab, the presence of ADAs is associated with low or undetectable serum drug concentrations, reduced clinical responses and injection site reactions[94]. Immunogenicity is lower with intravenous administration than with subcutaneous administration, and the use of background methotrexate improves serum concentrations of the drug. For example, following subcutaneous administration of golimumab, ADAs were detected in 5 out of 33 patients with RA compared with 1 out of 43 patients with ankylosing spondylitis when tested at 24 weeks[94].

In the AWARE trial, an observational study comparing golimumab with infliximab treatment in 1,270 patients with RA, 14.2% of the patients receiving infliximab and 3.9% of the patients receiving golimumab had infusion reactions. Rates of ADAs were higher in those receiving infliximab than in those receiving golimumab, irrespective of prior biologic exposure or methotrexate use[27].

Etanercept. Etanercept is a fusion protein of the p75 component of soluble TNF receptor 2 (TNFR2) and the IgG1 Fc region. The incidence of ADAs to etanercept is low, in part because many commercial assays are designed to assess the binding of ADAs to epitope-binding regions, which will not detect anti-linker-region ADAs[4]. A similar agent, lenercept, which is a fusion protein of the p55 component of soluble TNFR1 and the IgG1 Fc region, causes the formation of antibodies to two major linear epitopes located in close proximity to the linker region that can, with epitope spreading, yield anti-Fc region ADAs, which are associated with serum sickness; by contrast, no inhibition of epitope-binding regions was reported[95]. There has been at least one case of serum sickness associated with administration of etanercept to an adult patient with juvenile idiopathic arthritis (W. H. Robinson, personal communication). Immunogenicity data are also available for three etanercept biosimilars (TABLE 1). Overall, the incidence of ADAs is low, <10%, and all ADAs are non-neutralizing (some of which are transient); however, the association between ADAs and pharmacokinetics has not been investigated[6,96].

Lower serum drug concentrations are associated with the presence of ADAs and diminished clinical responses. In one study looking at the relationship between etanercept concentrations and clinical responses in patients with RA, the patients with lower serum concentrations of etanercept were predominantly women, had a higher BMI and glomerular filtration rate and were receiving lower doses of methotrexate than those patients with higher serum concentrations of the biologic agent[97]. However, no ADAs were detectable in the sera of these patients, which might otherwise have explained these findings. In another study involving 186 patients with RA, circulating concentrations of TNF increased in the patients following the administration of etanercept, similar to the effects seen with adalimumab[20]. Notably, in RCTs, etanercept combined with methotrexate therapy is more effective than etanercept monotherapy in patients with RA, regardless of the dose of methotrexate administered.

Certolizumab pegol. Certolizumab pegol is a $F(ab')^2$ fragment of a humanized anti-TNF antibody that is conjugated to PEG. In various RCTs of this drug in patients with RA or psoriasis, 3–37% of the patients developed ADAs (TABLE 1); the majority of the ADAs were neutralizing and were associated with lower serum drug concentrations and reduced efficacy[4,98]. In a study of 115 patients, ADA formation correlated inversely with serum drug concentrations (both measured in random samples rather than in trough blood samples) and higher concentrations of the biologic agent correlated with a good treatment response. In a smaller study of 40 patients with RA, 65% of the patients developed ADAs, but the presence of these antibodies did not seem to influence the circulating drug concentrations in these individuals (measured in trough serum samples). The presence of ADAs was associated with a reduction in drug concentrations over time; nevertheless, certolizumab pegol concentrations remain high in most ADA-positive patients. Furthermore, ex vivo, the TNF neutralization capacity of the patients' blood correlated with their serum drug concentrations but not with the formation of ADAs, potentially reflecting the presence of ADAs that recognize the PEG component of the drug[99]. Use of an initial loading dose of certolizumab pegol and concurrent methotrexate therapy helped to mitigate immunogenicity, regardless of the dose of methotrexate used.

Rituximab

As a B cell-depleting, chimeric anti-CD20 monoclonal antibody, the immunogenicity of rituximab is underestimated[4]. This therapy is administered intermittently, and repeated courses of rituximab, particularly in patients with autoimmune diseases such as RA, systemic lupus erythematosus and anti-neutrophil cytoplasmic antibody-associated vasculitis, can result in loss of response in some individuals, which can be recaptured using a humanized or fully human anti-CD20 monoclonal antibody[100]. As discussed earlier for infliximab, epitope mapping studies have revealed potentially immunogenic T cell epitopes in rituximab[75].

Background therapy with methotrexate and other anti-proliferative agents is associated with a lower incidence of ADAs to rituximab and a longer efficacy of treatment. However, studies of the effect of differing dose regimens on the immunogenicity of rituximab are lacking. Data are also available for the three rituximab biosimilars[3]; in RCTs of these biosimilars in RA, 0–21% of patients had ADAs following the second course of therapy (TABLE 1).

Abatacept

Abatacept is a cytotoxic T lymphocyte protein 4 (CTLA4)–Fc fusion protein, designed to inhibit T cell activation. The immunogenicity of abatacept has been

extensively studied in various RCTs in patients with RA, including studies comparing intravenous with subcutaneous administration, as well as studies of the effect of switching from intravenous to subcutaneous therapy or the effect of discontinuation and reinstitution of subcutaneous treatment (reviewed in detail elsewhere)[4]. As with etanercept, the immunogenic portion of abatacept is the linker between the CTLA4 extracellular domain and the IgG1 Fc region. In all the switching studies, <5% of the patients had ADAs following either intravenous or subcutaneous administration, switching or discontinuation and restart of therapy, and all of the antibodies were non-neutralizing.

IL-6 inhibitors

Tocilizumab. Tocilizumab is a humanized monoclonal antibody to the soluble IL-6 receptor (sIL-6R). As with abatacept, the immunogenicity of tocilizumab has been studied in RCTs comparing intravenous administration and subcutaneous administration in patients with RA[1,101]. The immunogenicity of subcutaneous and intravenous tocilizumab was similar when tested using a non-drug-tolerant assay with moderate sensitivity: 69 (1.2%) of the 5,875 patients treated with intravenous tocilizumab and 47 (1.5%) of the 3,099 treated with subcutaneous tocilizumab were ADA-positive; the majority of ADAs were neutralizing[101]. Anaphylaxis events can occur with intravenous therapy and were reported in 0.1% of patients with RA (3 of 2,644) in the 24-week results of RCTs of this therapy, and in 0.2% of patients (8 of 4,009), generally during the second to fourth infusions, in a study looking at long-term exposure[102]. Anaphylaxis also occurred in 1 patient (out of 56) in a trial of tocilizumab in patients with systemic juvenile idiopathic arthritis[103]. Furthermore, 4% of the patients receiving intravenous tocilizumab had infusion reactions and 10% of the patients receiving subcutaneous tocilizumab had injection site reactions[101].

Notably, drug reaction with eosinophilia and systemic symptoms syndrome was reported in a patient with adult-onset Still's disease following administration of 8 mg/kg intravenous tocilizumab[104]. The rash produced by the drug differed from the original rash caused by the disease, and a biopsy confirmed the presence of a lymphocytic and eosinophilic perivascular infiltrate, which was associated with a high peripheral eosinophil count and elevated liver function tests.

Sarilumab. Sarilumab is a fully human monoclonal antibody to sIL-6R, produced using homologous recombination. Using a sensitive assay (an ECLIA that included an acid dissociation step), ADAs were assessed in 132 patients with RA who were randomly assigned to receive 150 mg ($n=65$) or 200 mg ($n=67$) sarilumab every 2 weeks[105]. Persistent ADAs were detected in 12.3% and 6.1% of individuals receiving the 150 mg and 200 mg doses, respectively, of which 6.1% and 3.0% were neutralizing ADAs. A single hypersensitivity event of rash was reported and no incident anaphylaxis, and the presence of ADAs affected neither the efficacy nor the safety of the drug, which produced similar responses in ADA-positive and ADA-negative patients.

IL-12–IL-23 inhibitor

Ustekinumab is a human IgG1 monoclonal antibody to the p40 subunit of IL-12 and IL-23 that is approved for the treatment of psoriasis, PsA and Crohn's disease. Immunogenicity data are available from RCTs in psoriasis and PsA[1], as well as from a prospective observational study of ustekinumab in 76 patients with plaque psoriasis (in which serum concentrations of ADAs and ustekinumab were measured by radioimmunoassay and ELISA, respectively)[106]. In the latter study, after a mean of 13 months of treatment, ADAs were detectable in 6.5% of the patients, the presence of which were associated with significantly lower serum drug concentrations (0.01 mg/l versus 0.2 mg/l; $P<0.001$) and a reduced treatment response (as assessed by a 50% reduction in the psoriasis area and severity index score; 0% versus 69%; $P=0.004$). The percentages of ADA-positive patients were similar among those with prior exposure to adalimumab with and without anti-adalimumab antibodies (14.3% versus 12.5%; $P=1.00$).

Researchers have compared and validated different measurement approaches for the assessment of ustekinumab immunogenicity, following the recommendations of the EMA and FDA[107]; in this assessment, a newly developed ELISA-based acidification assay for detecting neutralizing ADAs was compared with surface plasmon resonance, a conventional ELISA and cell-based neutralization assays. The detection of ADAs was increased after the acidification step, indicating the release of ustekinumab from binding sites owing to the presence of neutralizing ADAs.

IL-17A inhibitors

Secukinumab. Secukinumab is a fully human monoclonal antibody that recognizes IL-17A and is approved for the treatment of psoriasis, PsA and SpA[1]. In RCTs, researchers have used ECLIAs to assess the immunogenicity of secukinumab (administered as monthly subcutaneous infusions with or without intravenous or subcutaneous loading doses) in PsA (the FUTURE 1–3 RCTs) and in SpA (the MEASURE 1–4 RCTs) at baseline and at weeks 16, 24 and 52 (REF.[108]). In the treatment groups, ADAs were detectable in 0.35% (5 of 1,414) of the patients with PsA and 0.69% (8 of 1,164) of the patients with SpA over 52 weeks; 2 of the 5 ADA-positive patients with PsA and 1 of the 8 ADA-positive patients with SpA had received concurrent methotrexate therapy. Only one of the patients had neutralizing ADAs, and the presence of ADAs was not associated with changes in serum drug concentrations, loss of efficacy or adverse events. Data from MHC-associated peptide proteomics analysis and T cell activation assays suggest that secukinumab is comparable to other fully human monoclonal antibodies with low immunogenicity with regard to the types of potential T cell epitopes and T cell response rates[109].

Ixekizumab. Ixekizumab is a humanized monoclonal antibody to IL-17A that is approved for the treatment of psoriasis, PsA and SpA. ADAs have been detected using a drug-tolerant affinity capture elution approach, in which ADA-positive patients were divided into

negative, low (<1:160), moderate (≥1:160 to <1:1,280) and high (≥1:1,280) titre groups[110]. In 385 patients with psoriasis who were treated for 60 weeks in a phase III RCT, 17.4% had detectable ADAs, only 3.5% of which were neutralizing ADAs. Some preliminary immunogenicity data on ixekizumab in PsA is available from the SPIRIT-P1 RCT (biologic-naive patients) and the SPIRIT-P2 RCT (patients who have an inadequate response or intolerance to TNF inhibition), in which patients received a 160-mg loading dose subcutaneously followed by 80 mg ixekizumab every 2 or 4 weeks[111]. Of the 223 patients from both RCTs being treated concomitantly with ixekizumab and methotrexate, ADAs were detectable in 11 (10.3%) and 6 (5.2%) of those on 4-week or 2-week dosing regimens, respectively, and of the 222 patients receiving ixekizumab monotherapy, ADAs were detectable in 13 (12%) and 9 (8.6%) of those on 4-week or 2-week dosing regimens, respectively[111]. As in psoriasis, the majority of the ADAs were present at low titres, and some were neutralizing, but ADA positivity did not have an effect on the long-term efficacy of the drug.

A sensitive T cell assay format has also been used to determine reactivity to secukinumab, ixekizumab and adalimumab in 16 healthy individuals[112]. Monocyte-derived dendritic cells from individuals with the most common HLA-DR alleles that occur in the ethnically mixed European population were incubated with either the individual monoclonal antibodies or with keyhole limpet haemocyanin (KLH) as a positive control. $CD4^+$ T cell lines were then generated in vitro by co-culture with the dendritic cells, the antigen specificity of the T cell lines tested by a type I interferon ELISpot assay and the mean frequency of antigen-specific cells per million donor cells determined. Responses were detected to KLH in all samples, whereas only 1 individual responded to secukinumab, 9 responded to ixekizumab and 9 responded to adalimumab, reflecting the lower immunogenicity of secukinumab compared with ixekizumab or adalimumab.

Switching studies

Several open label studies have investigated the effects of switching biologic therapies in patients with rheumatic diseases who do not respond, or who respond poorly, to a TNF inhibitor; these patients might either be switched to another TNF inhibitor or to a different class of biologic therapy, such as abatacept or rituximab. The results of the RESTART trial ($n = 197$) confirmed that patients who do not respond to either adalimumab or etanercept might respond to infliximab, with 52% of the switched patients achieving a EULAR clinical response at week 26 (REF.[113]). In a different cohort study investigating a switch to adalimumab in patients who did not respond to infliximab therapy ($n = 235$), patients with ADAs to infliximab developed non-cross-reactive ADAs to adalimumab more often than patients without ADAs to infliximab (27% versus 18%; $P = 0.039$); however, there was no difference in the changes in the 28-joint disease activity score between the two groups[79]. Thus, more ADA-positive individuals who switch therapy develop non-cross-reactive ADAs to a second or third TNF inhibitor than ADA-negative individuals who switch; however, many individuals with a secondary inadequate response show no evidence of immunogenicity. In a separate study, 89 individuals with a secondary inadequate response to adalimumab or infliximab were switched to etanercept and compared with 203 TNF inhibitor-naive patients[114]. There was no difference in responses between TNF inhibitor-naive and ADA-positive individuals who switched medication, whereas poorer responses were seen in those who switched medication and were ADA-negative, suggesting that inadequate responses to therapy in this latter group reflected synovitis that was no longer TNF dependent.

For 'non-medical' drug switching, for example, switching from intravenous to subcutaneous administration of the same biologic agent for convenience and/or cost reasons, the therapeutic responses before and after switching are usually comparable[115]. Non-medical switching to biosimilars has been reviewed extensively elsewhere[6] and is not a topic for this manuscript. As no biosimilar has been approved in the USA as an interchangeable product (a regulatory designation only available in the USA), switching between a reference product and a biosimilar is not currently relevant to clinical practice in the USA.

Clinical practice implications

Given the immunogenicity of TNF inhibitors, and its therapeutic consequences, researchers have advocated monitoring serum drug and ADA levels[116,117], although the cost-effectiveness of this practice has not yet been as robustly demonstrated for rheumatic diseases as it has for inflammatory bowel disease[118–120]. In theory, measurements of circulating drug concentrations could enable rheumatologists to personalize dosing, avoiding both under-exposure to the drug, which might reduce treatment efficacy, and over-exposure to the drug, which might increase the risk of adverse events, such as infections. Combined with ADA measurements, drug concentration measurements might also be helpful when assessing non-response to therapy (FIG. 4).

At a population level, an optimal blood concentration of a biologic agent theoretically exists that maintains the patients in sustained remission without leading to over-exposure to the drug. For example, with adalimumab, a trough concentration of 51 g/ml might be optimal[121]. Using this knowledge, it might then be possible to lengthen the intervals between doses for patients with high trough concentrations. Similar approaches could be developed for other biologic agents. Conversely, if patients fail to respond to a therapy, then knowledge of drug concentrations and ADAs can also be helpful. In individuals who exhibit a primary non-response to a therapy, a serum drug concentration measurement within the therapeutic range could suggest the need to switch to another class of drug, whereas low serum drug concentrations could suggest that a higher dose is necessary. Similar rules can apply for individuals with a secondary non-response to therapy, except that the presence of ADAs alongside low serum drug concentrations might suggest that an alternative therapy from the same biologic class be

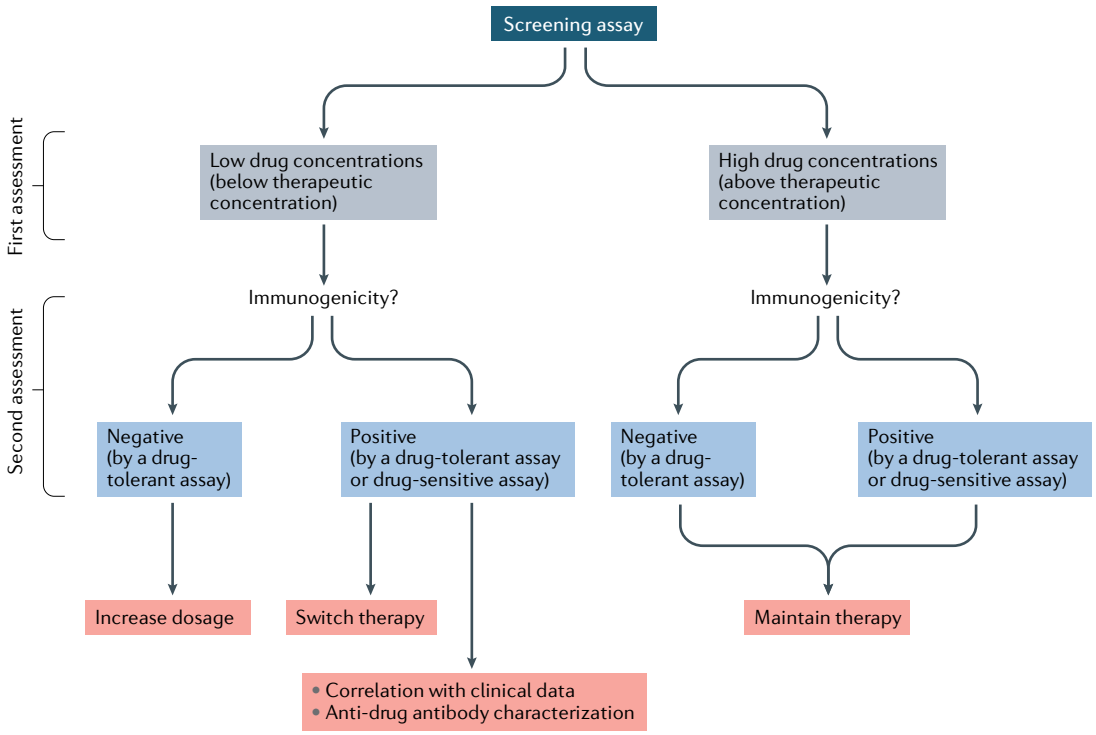


Fig. 4 | **Therapeutic drug monitoring strategies.** A potential therapeutic drug monitoring decision algorithm that integrates information regarding serum drug concentrations and immunogenic responses and that could be used in the assessment of patients with rheumatoid arthritis being treated with TNF inhibitors. The algorithm also illustrates how the assays can potentially help to guide treatment strategy. For example, if loss of efficacy of an anti-TNF monoclonal antibody is associated with the development of anti-drug antibodies, then a different TNF inhibitor might be effective. However, if loss of efficacy is not associated with anti-drug antibody development, then the best strategy might be to switch to a different therapeutic class.

selected, whereas low serum drug concentrations in the absence of ADAs might suggest poor adherence, assuming that the drug was previously effective[89,93]. Despite these theoretical benefits, preliminary results from the NOR-DRUM study revealed no clinical benefits of therapeutic drug monitoring in patients initiating infliximab therapy across a variety of inflammatory diseases[122]; no clinical differences were observed between the therapeutic drug monitoring group (consisting of individualized therapy with infliximab according to serum drug concentrations and ADA status) or the control group (administration of infliximab without knowledge of the serum drug concentrations or ADA status) after 30 weeks of treatment. However, the study did not specify how often the dose was adjusted in the therapeutic drug monitoring group, which would have been useful to know, particularly for those patients with sub-optimal serum drug concentrations. In terms of adverse effects, the development of infusion reactions with infliximab in the presence of ADAs should prompt a change of treatment, potentially to another TNF inhibitor. However, only a minority of patients with ADAs develop infusion reactions. Injection site reactions might reflect immunogenicity but can also be attributed to the formulation of the agent in use.

Without therapeutic drug monitoring, the decision to switch to a different therapy following a primary or secondary inadequate response depends on the clinician's inclination and the preference of the hospital. Therapeutic drug monitoring could improve this procedure by identifying subgroups of patients who might profit from switching to either a second TNF inhibitor or to a biologic of a different class. For example, loss of clinical response to a first TNF inhibitor in the absence of ADAs is predictive of a potential lack of response if switched to a second TNF inhibitor[123]. However, in a series of 137 patients with RA, neither the ADA status nor the serum drug concentrations were predictive of subsequent responses to TNF inhibitors or to other classes of biologic agents in patients who were not responsive to adalimumab therapy[124]. Notably, this study was a retrospective analysis of patient data rather than a prospective trial, and used random samples rather than trough blood samples to measure drug concentrations and ADAs. Importantly, clinical monitoring is adequate on its own in rheumatology, unlike other specialties, which might require additional invasive tests.

Although a number of algorithms have been developed for therapeutic drug monitoring of biologic agents, a major remaining question relates to cost-effectiveness. Implementation of therapeutic drug monitoring will potentially require additional hospital attendance by patients to measure trough drug concentrations (for self-injected medications), laboratory set-up and standardization and an interpretation service, in addition to the financial costs of the assays. Although therapeutic drug monitoring has become the preferred practice for

the treatment of inflammatory bowel disease in the USA, there is a dearth of evidence as to whether therapeutic drug monitoring improves clinical outcomes and, particularly, whether this approach can be cost effective[125]. Consequently, the UK National Institute for Health and Care Excellence does not recommend routine therapeutic drug monitoring in patients with either RA or Crohn's disease but does recommend further research in this area[126]. By contrast, although limited evidence was available, a systematic review of studies in patients with inflammatory bowel disease suggests that this approach has cost-saving benefits (particularly for reactive therapeutic drug monitoring), as well as potential benefits in terms of improving TNF inhibitor durability (particularly for proactive therapeutic drug monitoring)[127]. Importantly, if therapeutic drug monitoring does become a cost-effective addition to the care of patients receiving biologic agents in rheumatology, education for health-care professionals and patients would be required concerning the different types of assay platforms available, their standardization and their interpretation.

Conclusions

The advent of biosimilars and the need for rigorous regulatory standards have catalysed research and innovation in the measurement of immunogenicity, resulting in a better understanding of its determinants, consequences and clinical implications. Furthermore, a variety of methods now exist for characterizing ADAs, which have highlighted differences in immunogenicity among different biologic agents. Although the measurement of circulating biologic drug concentrations in concert with ADA measurements can, in theory, optimize dosing strategies, the attractiveness of therapeutic drug monitoring is not yet supported by high-quality cost-effectiveness studies, which will be required before such testing becomes a part of standard care. Additionally, the simple methods for therapeutic drug monitoring that have appeared in the literature should not detract from the sophistication of the assays used, which demand a degree of interpretation by the requesting clinicians, as well as education of the patients themselves.

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1. Isaacs, J. D. et al. Humanised monoclonal antibody therapy for rheumatoid arthritis. *Lancet* **340**, 748–752 (1992).
2. Dörner, T. et al. The role of biosimilars in the treatment of rheumatic diseases. *Ann. Rheum. Dis.* **72**, 322–328 (2013).
3. Dörner, T. et al. The changing landscape of biosimilars in rheumatology. *Ann. Rheum. Dis.* **75**, 974–982 (2016).
4. Strand, V. et al. Immunogenicity of biologics in chronic inflammatory diseases: a systematic review. *BioDrugs* **31**, 299–316 (2017).
5. Kalden, J. R. & Schulze-Koops, H. Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment. *Nat. Rev. Rheumatol.* **13**, 707–718 (2017).
6. Strand, V. et al. Immunogenicity of biosimilars for rheumatic diseases, plaque psoriasis, and inflammatory bowel disease: a review from clinical trials and regulatory documents. *BioDrugs* **34**, 27–37 (2020).
7. Rup, B. et al. Standardizing terms, definitions and concepts for describing and interpreting unwanted immunogenicity of biopharmaceuticals: recommendations of the innovative medicines initiative ABIRISK consortium. *Clin. Exp. Immunol.* **181**, 385–400 (2015).
8. Pyzik, M. et al. The neonatal Fc receptor (FcRn): A misnomer? *Front. Immunol.* **10**, 1540 (2019).
9. Schellekens, H. Bioequivalence and the immunogenicity of biopharmaceuticals. *Nat. Rev. Drug Discov.* **6**, 457–462 (2002).
10. Montes, A. et al. Rheumatoid arthritis response to treatment across IgG1 allotype–anti-TNF incompatibility: a case-only study. *Arthritis Res. Ther.* **17**, 63 (2015).
11. Ratanji, K. D., Derrick, J. P., Dearman, R. J. & Kimber, I. Immunogenicity of therapeutic proteins: influence of aggregation. *J. Immunotoxicol.* **11**, 99–109 (2014).
12. Gill, K. L., Machavaram, K. K., Rose, R. H. & Chetty, M. Potential sources of inter-subject variability in monoclonal antibody pharmacokinetics. *Clin. Pharmacokinet.* **55**, 789–805 (2016).
13. Carmona, L., Gómez-Reino, J. J. & BIOBADASER group. Survival of TNF antagonists in spondylarthritis is better than in rheumatoid arthritis. Data from the Spanish registry BIOBADASER. *Arthritis Res. Ther.* **8**, R72 (2006).
14. Fafá, B. P. et al. Drug survival and causes of discontinuation of the first anti-TNF in ankylosing spondylitis compared with rheumatoid arthritis: analysis from BIOBADARASIL. *Clin. Rheumatol.* **34**, 921–927 (2015).
15. Park, W. et al. A randomised, double-blind, multicentre, parallel-group, prospective study comparing the pharmacokinetics, safety, and efficacy of CT-P13 and innovator infliximab in patients with ankylosing spondylitis: the PLANETAS study. *Ann. Rheum. Dis.* **72**, 1605–1612 (2013).
16. Ungar, B. et al. Ashkenazi Jewish origin protects against formation of antibodies to infliximab and therapy failure. *Medicine* **94**, e673 (2015).
17. Atiqi, S., Hooijberg, F., Loeff, F. C., Rispens, T. & Wolbink, G. J. Immunogenicity of TNF-inhibitors. *Front. Immunol.* **11**, 312 (2020).
18. Berkhout, L. C. et al. Dynamics of circulating TNF during adalimumab treatment using a drug-tolerant TNF assay. *Sci. Transl. Med.* **11**, eaat3356 (2019).
19. van Schie, K. A. et al. Therapeutic TNF inhibitors can differentially stabilize trimeric TNF by inhibiting monomer exchange. *Sci. Rep.* **6**, 32747 (2016).
20. Berkhout, L. C. et al. The effect of methotrexate on tumour necrosis factor concentrations in etanercept-treated rheumatoid arthritis patients. *Rheumatology* **59**, 1703–1708 (2019).
21. Benjamin, R. J., Cobbold, S. P., Clark, M. R. & Waldmann, H. Tolerance to rat monoclonal antibodies. Implications for serotherapy. *J. Exp. Med.* **163**, 1539–1552 (1986).
22. Isaacs, J. D. & Waldmann, H. Helplessness as a strategy for avoiding antiglobulin responses to therapeutic monoclonal antibodies. *Ther. Immunol.* **1**, 303–312 (1994).
23. Gilliland, L. K. et al. Elimination of the immunogenicity of therapeutic antibodies. *J. Immunol.* **162**, 3663–3671 (1999).
24. Jefferis, R. & Lefranc, M.-P. Human immunoglobulin allotypes: possible implications for immunogenicity. *MAbs* **1**, 332–338 (2009).
25. Webster, C. I. et al. A comparison of the ability of the human IgG1 allotypes G1m3 and G1m1,17 to stimulate T-cell responses from allotype matched and mismatched donors. *MAbs* **8**, 253–263 (2016).
26. Rebello, P. R., Hale, G., Friend, P. J., Cobbold, S. P. & Waldmann, H. Anti-globulin responses to rat and humanized CAMPATH-1 monoclonal antibody used to treat transplant rejection. *Transplantation* **68**, 1417–1420 (1999).
27. Schwartzman, S. et al. United States rheumatology practice-based real-world evidence of infusion reactions in rheumatoid arthritis patients treated with intravenous golimumab or infliximab: impact of prior biologic exposure and methotrexate utilization [abstract]. *Ann. Rheum. Dis.* **79**, 994 (2020).
28. Wang, J. et al. Neutralizing antibodies to therapeutic enzymes: considerations for testing, prevention and treatment. *Nat. Biotechnol.* **26**, 901–908 (2008).
29. Bali, D. S. et al. Predicting cross-reactive immunological material (CRIM) status in Pompe disease using GAA mutations: lessons learned from 10 years of clinical laboratory testing experince. *Am. J. Med. Genet. C Semin. Med. Genet.* **160C**, 40–49 (2012).
30. Garman, R. D., Munroe, K. & Richards, S. M. Methotrexate reduces antibody responses to recombinant human alpha-galactosidase a therapy in a mouse model of Fabry disease. *Clin. Exp. Immunol.* **137**, 496–502 (2004).
31. Joseph, A., Munroe, K., Housman, M., Garman, R. & Richards, S. Immune tolerance induction to enzyme-replacement therapy by co administration of short-term, low-dose methotrexate in a murine Pompe disease model. *Clin. Exp. Immunol.* **152**, 138–146 (2008).
32. Joseph, A. et al. Transient low-dose methotrexate induces tolerance to murine anti-thymocyte globulin and together they promote long-term allograft survival. *J. Immunol.* **189**, 732–743 (2012).
33. Gupta, S. et al. Association of immune response with efficacy and safety outcomes in adults with phenylketonuria administered pegvaliase in phase 3 clinical trials. *EBioMedicine.* **37**, 366–373 (2018).
34. Sundy, J. S. et al. Efficacy and tolerability of pegloticase for the treatment of chronic gout in patients refractory to conventional treatment: two randomized controlled trials. *JAMA* **306**, 711–720 (2011).
35. Baraf, H. S. et al. Tophus burden reduction with pegloticase: results from phase 3 randomised trials and open-label extension in patients with chronic gout refractory to conventional therapy. *Arthritis Res. Ther.* **15**, R137 (2013).
36. Baraf, H. S., Yood, R. A., Ottery, F. D., Sundy, J. S. & Becker, M. A. Infusion-related reactions with pegloticase, a recombinant uricase for the treatment of chronic gout refractory to conventional therapy. *J. Clin. Rheumatol.* **20**, 427–432 (2014).
37. Keenan, R. T., Baraf, H. S. B. & LaMoreaux, B. Use of pre-infusion serum uric acid levels as a biomarker for infusion reaction risk in patients on pegloticase. *Rheumatol. Ther.* **6**, 299–304 (2019).
38. Lipsky, P. E. et al. Pegloticase immunogenicity: the relationship between efficacy and antibody development in patients treated for refractory chronic gout. *Arthritis Res. Ther.* **16**, R60 (2014).
39. Hershfield, M. S. et al. Induced and pre-existing anti-polyethylene glycol antibody in a trial of every 3-week dosing of pegloticase for refractory gout, including in organ transplant recipients. *Arthritis Res. Ther.* **16**, R63 (2014).
40. Bessen, S. Y., Bessen, M. Y. & Yung, C. M. Recapture and improved outcome of pegloticase response with methotrexate — a report of two cases and review of the literature. *Semin. Arthritis Rheum.* **49**, 56–61 (2019).
41. Botson, J. & Peterson, J. Pretreatment and co-administration with methotrexate improved durability of pegloticase response: a prospective, observational, proof-of-concept, case series. *J. Clin. Rheumatol.* https://doi.org/10.1097/RHU.0000000000001639 (2020).

42. Bessen, M. Y., Bessen, S. Y. & Yung, C. M. Concomitant immunosuppressant use with pegloticase in patients with tophaceous gout — a case series. *Int. J. Clin. Rheumatol.* **14**, 238–245 (2019).
43. Rainey, H., Baraf, H. S. B., Yeo, A. & Lipsky, P. Companion immunosuppression with azathioprine increases the frequency of persistent responsiveness to pegloticase in patients with chronic refractory gout [abstract]. *Ann. Rheum. Dis.* **79**, 442–443 (2020).
44. Botson, J. et al. Pegloticase response improvement by co-treatment with methotrexate: results from the MIRROR open label clinical trial in patients with uncontrolled gout [abstract]. *Ann. Rheum. Dis.* **79**, 446 (2020).
45. Masri, K., Winterling, K. & Lamoreaux, B. Leflunomide co-therapy with pegloticase in uncontrolled gout [abstract]. *Ann. Rheum. Dis.* **79**, 454 (2020).
46. Kishimoto, T. K. Development of ImmTOR tolerogenic nanoparticles for the mitigation of anti-drug antibodies. *Front. Immunol.* **11**, 969 (2020).
47. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03905512 (2020).
48. Krishna, M. & Nadler, S. G. Immunogenicity to biotherapeutics — the role of anti-drug immune complexes. *Front. Immunol.* **7**, 21 (2016).
49. van Schie, K. A. et al. Restricted immune activation and internalisation of anti-idiotype complexes between drug and antidrug antibodies. *Ann. Rheum. Dis.* **77**, 1471–1479 (2018).
50. Lockwood, C. M., Thiru, S., Isaacs, J. D., Hale, G. & Waldmann, H. Long-term remission of intractable systemic vasculitis with monoclonal antibody therapy. *Lancet* **341**, 1620–1622 (1993).
51. Bivi, N. et al. Investigation of pre-existing reactivity to biotherapeutics can uncover potential immunogenic epitopes and predict immunogenicity risk. *MAbs* **11**, 861–869 (2019).
52. Maini, R. N. et al. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum.* **41**, 1552–1563 (1998).
53. Hernandez-Florez, D. et al. Comparison of two ELISA versions for infliximab serum levels in patients diagnosed with ankylosing spondylitis. *Rheumatol. Int.* **35**, 1021–1025 (2015).
54. Steenholdt, C., Bendtzen, K., Brynskov, J., Thomsen, O. Ø. & Ainsworth, M. A. Clinical implications of measuring drug and anti-drug antibodies by different assays when optimizing infliximab treatment failure in Crohn's disease: post hoc analysis of a randomized controlled trial. *Am. J. Gastroenterol.* **109**, 1055–1064 (2014).
55. Cohen, H. P. et al. Switching reference medicines to biosimilars: a systematic literature review of clinical outcomes. *Drugs* **78**, 463–478 (2018).
56. European Medicines Agency. Guideline on immunogenicity assessment of therapeutic proteins. *EMA* https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-immunogenicity-assessment-therapeutic-proteins-revision-1_en.pdf (2017).
57. US Department of Health and Human Services. Immunogenicity testing of therapeutic protein products — developing and validating assays for anti-drug antibody detection. Guidance for industry. *FDA* https://www.fda.gov/regulatory-information/search-fda-guidance-documents/immunogenicity-testing-therapeutic-protein-products-developing-and-validating-assays-anti-drug (2019).
58. Bloem, K. et al. Systematic comparison of drug-tolerant assays for anti-drug antibodies in a cohort of adalimumab-treated rheumatoid arthritis patients. *J. Immunol. Methods* **418**, 29–38 (2015).
59. Bader, L. I. et al. Assays for infliximab drug levels and antibodies: a matter of scales and categories. *Scand. J. Immunol.* **86**, 165–170 (2017).
60. Bendtzen, K. Immunogenicity of anti-TNF-α biotherapies. II. Clinical relevance of methods used for anti-drug antibody detection. *Front. Immunol.* **6**, 109 (2015).
61. Cobbold, S. P., Rebello, P. R., Davies, H. F., Friend, P. J. & Clark, M. R. A simple method for measuring patient anti-globulin responses against isotypic or idiotypic determinants. *J. Immunol. Methods* **127**, 19–24 (1990).
62. van Schouwenburg, P. A., Rispens, T. & Wolbink, G. J. Immunogenicity of anti-TNF biologic therapies for rheumatoid arthritis. *Nat. Rev. Rheumatol.* **9**, 164–172 (2013).
63. Liang, M. et al. Detection of high- and low-affinity antibodies against a human monoclonal antibody using various technology platforms. *Assay Drug Dev. Technol.* **5**, 655–662 (2007).
64. Zhong, Z. D. et al. Drug target interference in immunogenicity assays: recommendations and mitigation strategies. *AAPS J.* **19**, 1564–1575 (2017).
65. Jani, M. et al. Clinical utility of random anti-tumor necrosis factor drug-level testing and measurement of antidrug antibodies on the long-term treatment response in rheumatoid arthritis. *Arthritis. Rheum.* **67**, 2011–2019 (2015).
66. Dirks, N. L. & Meibohm, B. Population pharmacokinetics of therapeutic monoclonal antibodies. *Clin. Pharmacokinet.* **49**, 633–659 (2010).
67. Wolbink, G. J., Aarden, L. A. & Dijkmans, B. A. C. Dealing with immunogenicity of biologicals: assessment and clinical relevance. *Curr. Opin. Rheumatol.* **21**, 211–215 (2009).
68. Bloem, K., Hernández-Breijo, B., Martínez-Feito, A. & Rispens, T. Immunogenicity of therapeutic antibodies: monitoring antidrug antibodies in a clinical context. *Ther. Drug Monit.* **39**, 327–332 (2017).
69. Ternant, D., Bejan-Angoulvant, T., Passot, C., Mulleman, D. & Paintaud, G. Clinical pharmacokinetics and pharmacodynamics of monoclonal antibodies approved to treat rheumatoid arthritis. *Clin. Pharmacokinet.* **54**, 1107–1123 (2015).
70. Gunn, G. R. 3rd et al. From the bench to clinical practice: understanding the challenges and uncertainties in immunogenicity testing for biopharmaceuticals. *Clin. Exp. Immunol.* **184**, 137–146 (2016).
71. Benucci, M. et al. Laboratory monitoring of biological therapies in rheumatology: the role of immunogenicity. *Ann. Lab. Med.* **40**, 101–113 (2020).
72. Gorovits, B. et al. Immunoassay methods used in clinical studies for the detection of anti-drug antibodies to adalimumab and infliximab. *Clin. Exp. Immunol.* **192**, 348–365 (2018).
73. Freeman, K. et al. Test accuracy of drug and antibody assays for predicting response to antitumor necrosis factor treatment in Crohn's disease: a systematic review and meta-analysis. *BMJ Open* **7**, e014581 (2017).
74. Goncalves, J. et al. Antigenic response to CT-P13 and infliximab originator in inflammatory bowel disease patients shows similar epitope recognition. *Aliment. Pharmacol. Ther.* **48**, 507–522 (2018).
75. Hamze, M. et al. Characterization of CD4 T cell epitopes of infliximab and rituximab identified from healthy donors. *Front. Immunol.* **8**, 500 (2017).
76. Mahler, S. M., Marquis, C. P., Brown, G., Roberts, A. & Hoogenboom, H. R. Cloning and expression of human V-genes derived from phage display libraries as fully assembled human anti-TNF alpha monoclonal antibodies. *Immunotechnology* **3**, 31–43 (1997).
77. [No authors listed] Nobel work that galvanized an industry. *Nat Biotechnol.* **36**, 1023 (2018).
78. Harding, F. A., Stickler, M. M., Razo, J. & DuBridge, R. B. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *MAbs* **2**, 256–265 (2010).
79. Bartelds, G. M. et al. Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-tumour necrosis factor naive patients: a cohort study. *Ann. Rheum. Dis.* **69**, 817–821 (2010).
80. Korswagen, L. A. et al. Venous and arterial thromboembolic events in adalimumab-treated patients with anti-adalimumab antibodies: a case series and cohort study. *Arthritis Rheum.* **63**, 877–883 (2011).
81. Bartelds, G. M. et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA* **305**, 1460–1468 (2011).
82. van Schouwenburg, P. A. et al. Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralisation. *Ann. Rheum. Dis.* **72**, 104–109 (2013).
83. Vogelzang, E. H. et al. Anti-adalimumab antibodies and adalimumab concentrations in psoriatic arthritis: an association with disease activity at 28 and 52 weeks follow-up. *Ann. Rheum. Dis.* **73**, 2178–2182 (2014).
84. Kneepkens, E. L. et al. Immunogenicity, adalimumab levels and clinical response in ankylosing spondylitis patients during 24 weeks of follow-up. *Ann. Rheum. Dis.* **74**, 396–401 (2015).
85. Pouw, M. F. et al. Key findings towards optimising adalimumab treatment: the concentration-effect curve. *Ann. Rheum. Dis.* **74**, 513–518 (2015).
86. Bitoun, S. et al. Methotrexate and BAFF interaction prevents immunization against TNF inhibitors. *Ann. Rheum. Dis.* **77**, 1463–1470 (2018).
87. Docourau, E. et al. Methotrexate effect on immunogenicity and long-term maintenance of adalimumab in axial spondyloarthritis: a multicentric randomised trial. *RMD Open* **6**, e001047 (2020).
88. Humira® (adalimumab) US Package Insert (AbbVie Inc., 2008).
89. Burmester, G. R. et al. Efficacy and safety of ascending methotrexate dose in combination with adalimumab: the randomised CONCERTO trial. *Ann. Rheum. Dis.* **74**, 1037–1044 (2015).
90. Deng, Y. et al. Methotrexate reduces the clearance of adalimumab by increasing the concentration of neonatal Fc receptor in tissues. *Pharm. Res.* **36**, 157 (2019).
91. Krieckaert, C. L., Nurmohamed, M. T. & Wolbink, G. J. Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. *Ann. Rheum. Dis.* **71**, 1914–1915 (2012).
92. Dervieux, T., Kremer, J. M. & Weinblatt, M. E. Differing contribution of methotrexate polyglutamates to adalimumab blood levels as compared with etanercept. *Ann. Rheum. Dis.* **78**, 1285–1286 (2019).
93. Keizer, R. J., Huitema, A. D. R., Schellens, J. H. M. & Beijnen, J. H. Clinical pharmacokinetics of therapeutic monoclonal antibodies. *Clin. Pharmacokinet.* **49**, 493–507 (2010).
94. Chen, D.-Y. et al. Immunogenicity, drug trough levels and therapeutic response in patients with rheumatoid arthritis or ankylosing spondylitis after 24-week golimumab treatment. *Ann. Rheum. Dis.* **74**, 2261–2264 (2015).
95. Christen, U., Thuerkauf, R., Stevens, R. & Lesslauer, W. Immune response to a recombinant human TNFR55-IgG1 fusion protein: auto-antibodies in rheumatoid arthritis (RA) and multiple sclerosis (MS) patients have neither neutralizing nor agonist activities. *Hum. Immunol.* **60**, 774–790 (1999).
96. Moots, R. J. et al. The impact of anti-drug antibodies on drug concentrations and clinical outcomes in rheumatoid arthritis patients treated with adalimumab, etanercept, or infliximab: results from a multinational, real-world clinical practice, non-interventional study. *PLoS ONE* **12**, e0175207 (2017).
97. Jamnitski, A. et al. Patients non-responding to etanercept obtain lower etanercept concentrations compared with responding patients. *Ann. Rheum. Dis.* **71**, 88–91 (2012).
98. Jani, M. et al. High frequency of antidrug antibodies and association of random drug levels with efficacy in certolizumab pegol-treated patients with rheumatoid arthritis: results from the BRAGGSS cohort. *Ann. Rheum. Dis.* **76**, 208–213 (2017).
99. Berkhout, L. C. et al. The effect of certolizumab drug concentration and anti-drug antibodies on TNF neutralisation. *Clin. Exp. Rheum.* **38**, 306–313 (2020).
100. Yusof, M. Y. M. et al. Predicting and managing primary and secondary non-response to rituximab using B-cell biomarkers in systemic lupus erythematosus. *Ann. Rheum. Dis.* **76**, 1829–1836 (2017).
101. Burmester, G. R. et al. Low immunogenicity of tocilizumab in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **76**, 1078–1085 (2017).
102. Actemra® (tocilizumab) US Package Insert (Genentech Inc., 2013).
103. Yakota, S. et al. Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. *Lancet* **371**, 998–1006 (2008).
104. Zuelgaray, E., Domont, F., Peiffer-Smadja, N., Saadoun, D. & Cacoub, P. Tocilizumab-induced drug reaction with eosinophilia and systemic symptoms syndrome in adult-onset Still disease: a case report. *Ann. Intern. Med.* **167**, 141–142 (2017).
105. Wells, A. F. et al. Immunogenicity of sarilumab monotherapy in patients with rheumatoid arthritis who were inadequate responders or intolerant to disease-modifying antirheumatic drugs. *Rheumatol. Ther.* **6**, 339–352 (2019).

106. Chiu, H.-Y., Chu, T. W., Cheng, Y.-P. & Tsai, T.-F. The association between clinical response to ustekinumab and immunogenicity to ustekinumab and prior adalimumab. *PLoS ONE* **10**, e0142930 (2015).
107. Mojtahed Poor, S. et al. Immunogenicity assay development and validation for biological therapy as exemplified by ustekinumab. *Clin. Exp. Immunol.* **196**, 259–275 (2019).
108. Deodar, A. et al. Secukinumab immunogenicity over 52 weeks in patients with psoriatic arthritis and ankylosing spondylitis. *J. Rheumatol.* **47**, 539–547 (2020).
109. Karle, A., Spindeldreher, S. & Kolbinger, F. Secukinumab, a novel anti-IL-17A antibody, shows low immunogenicity potential in human in vitro assays comparable to other marketed biotherapeutics with low clinical immunogenicity. *MAbs* **8**, 536–550 (2016).
110. Muram, T. M. et al. A highly sensitive and drug-tolerant anti-drug antibody screening assay for ixekizumab using affinity capture elution. *J. Invest. Dermatol.* **136**, 1513–1515 (2016).
111. Ritchlin, C. T., Merola, J. F., Gellet, A. M., Lin, C.-Y. & Muram, T. Anti-drug antibodies, efficacy, and impact of concomitant methotrexate in ixekizumab-treated patients with psoriatic arthritis [abstract]. *Arthritis Rheumatol.* **70**, 2576 (2018).
112. Spindeldreher, S. et al. Secukinumab demonstrates significantly lower immunogenicity potential compared to ixekizumab. *Dermatol. Ther.* **8**, 57–68 (2018).
113. Fleischmann, R. et al. Infliximab efficacy in rheumatoid arthritis after an inadequate response to etanercept or adalimumab: results of a target-driven active switch study. *Curr. Med. Res. Opin.* **30**, 2139–2149 (2014).
114. Jamnitski, A. et al. The presence or absence of antibodies to infliximab or adalimumab determines the outcome of switching to etanercept. *Ann. Rheum. Dis.* **70**, 284–288 (2011).
115. Reynolds, A., Koenig, A. S., Bananis, E. & Singh, A. When is switching warranted among biologic therapies in rheumatoid arthritis? *Expert Rev. Pharmacoecon. Outcomes Res.* **12**, 319–333 (2012).
116. Vincent, F. B. et al. Antidrug antibodies (ADAb) to tumour necrosis factor (TNF)-specific neutralising agents in chronic inflammatory diseases: a real issue, a clinical perspective. *Ann. Rheum. Dis.* **72**, 165–178 (2013).
117. Schaeverbeke, T. et al. Immunogenicity of biologic agents in rheumatoid arthritis patients: lessons for clinical practice. *Rheumatology* **55**, 210–220 (2016).
118. Bendtzen, K. Is there a need for immunopharmacologic guidance of anti-tumor necrosis factor therapies? *Arthritis Rheum.* **63**, 867–870 (2011).
119. Garcês, S. et al. A preliminary algorithm introducing immunogenicity assessment in the management of patients with RA receiving tumour necrosis factor inhibitor therapies. *Ann. Rheum. Dis.* **73**, 1138–1143 (2014).
120. Jani, M. et al. A microcosting study of immunogenicity and tumour necrosis factor alpha inhibitor drug level tests for therapeutic drug monitoring in clinical practice. *Rheumatology* **55**, 2131–2137 (2016).
121. l'Ami, M. J. et al. Successful reduction of overexposure in patients with rheumatoid arthritis with high serum adalimumab concentrations: an open-label, non-inferiority, randomised clinical trial. *Ann. Rheum. Dis.* **77**, 484–487 (2018).
122. Syversen, S. W. et al. Therapeutic drug monitoring compared to standard treatment of patients starting infliximab therapy: results from a multicentre randomised trial of 400 patients [abstract]. *Ann. Rheum. Dis.* **79**, 12 (2020).
123. Quistrebert, J. et al. Incidence and risk factors for adalimumab and infliximab anti-drug antibodies in rheumatoid arthritis: a European retrospective multicohort analysis. *Semin. Arthritis Rheum.* **48**, 967–975 (2019).
124. Ulijn, E. et al. Therapeutic drug monitoring of adalimumab in RA: no predictive value of adalimumab serum levels and anti-adalimumab antibodies for prediction of response to the next bDMARD. *Ann. Rheum. Dis.* **79**, 867–873 (2020).
125. National Institute for Health and Care Excellence. Therapeutic monitoring of TNF-alpha inhibitors in rheumatoid arthritis. Diagnostics guidance [DG36]. *NICE* https://www.nice.org.uk/guidance/dg36/chapter/1-Recommendations (2019).
126. National Institute for Health and Care Excellence. Therapeutic monitoring of TNF-alpha inhibitors in Crohn's disease (LISA-TRACKER ELISA kits, IDKmonitor ELISA kits, and Promonitor ELISA kits). Diagnostics guidance [DG22]. *NICE* https://www.nice.org.uk/guidance/dg22/chapter/1-Recommendations (2016).
127. Ricciuto, A., Dhaliwal, J., Walters, T. D., Griffiths, A. M. & Church, P. C. Clinical outcomes with therapeutic drug monitoring in inflammatory bowel disease: a systematic review with meta-analysis. *J. Crohns Colitis.* **12**, 1302–1315 (2018).
128. Tracey, D., Klareskog, L., Sasso, E. H., Salfeld, J. G. & Tak, P. P. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol. Ther.* **117**, 244–279 (2008).

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Toll-like receptor signalling in B cells during systemic lupus erythematosus

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Abstract | B lymphocytes have a central role in autoimmune diseases, which are often defined by specific autoantibody patterns and feature a loss of B cell tolerance. A prototypic disease associated with B cell hyperactivity is systemic lupus erythematosus (SLE). In patients with SLE, the loss of B cell tolerance to autoantigens is controlled in a cell-intrinsic manner by Toll-like receptors (TLRs), which sense nucleic acids in endosomes. TLR7 drives the extrafollicular B cell response and the germinal centre reaction that are involved in autoantibody production and disease pathogenesis. Surprisingly, TLR9 seems to protect against SLE, even though it is required for the production of autoantibodies recognizing double-stranded DNA-associated antigens, which are abundant in SLE and are a hallmark of this disease. The protective function of TLR9 is at least partly mediated by its capacity to limit the stimulatory activity of TLR7. The roles of TLR7 and TLR9 in the effector function of B cells in lupus-like disease and in patients with SLE, and the unique features of TLR signalling in B cells, suggest that targeting TLR signalling in SLE might be therapeutically beneficial.

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The diagnosis of autoimmune diseases often relies on the identification of characteristic autoantibody profiles, emphasizing their association with the activation of autoreactive B cells. Furthermore, B cell depletion therapy can have beneficial effects in patients with these disorders[1], highlighting the importance of B cells in the pathogenesis of autoimmune diseases. In autoimmune diseases B cells have been regarded almost exclusively for their role in autoantibody production, although we now know that they also mediate deleterious functions through antibody-independent activities, including: the presentation of antigen to T cells, co-stimulatory functions via the expression of accessory molecules engaging stimulatory receptors on T cells and the production of cytokines[2]. These findings highlight the need to extend the repertoire of effector B cell subsets studied with regard to autoimmune diseases beyond antibody-secreting plasmablasts and plasma cells.

Identifying the signalling pathways controlling the differentiation of effector B cell subsets might shed light on the pathophysiological mechanisms at play during autoimmune diseases. B cell activation is controlled by four classes of receptors, namely B cell receptors (BCRs) that bind autoantigens, cytokine receptors, receptors implicated in cognate T cell–B cell interactions (including checkpoint molecules), and innate immune receptors including Toll-like receptors (TLRs). The implication of TLRs in some autoimmune diseases is underlined by their association with polymorphisms in *TLR* genes (for example, *TLR4* and *TLR7*)[3,4]. TLR signalling promotes three key activities through which B cells can contribute to autoimmune diseases: the production of antibodies, the presentation of antigens to T cells and the production of cytokines[5–7]. The importance of both B cells and TLRs in autoimmune diseases suggests a role for intrinsic TLR signalling in B cells in these disorders.

In this Review we discuss how intrinsic TLR signalling controls the differentiation of effector B cells during autoimmune diseases, with a particular focus on systemic lupus erythematosus (SLE). First, we document the importance of intrinsic TLR signalling in B cells in the development of SLE. Second, we consider the contribution of intrinsic TLR signalling to the generation of pathogenic effector B cell subsets. Third, we highlight features of TLR signalling that are specific to B cells, some of which regulate their anti-inflammatory functions. Fourth, we discuss current therapeutic opportunities and perspectives for targeting TLR signalling in autoimmune diseases.

TLR signalling in B cells drives SLE

TLR7 predisposes humans to SLE. Genetic association studies implicate TLR signalling in SLE[8–10]. In particular, polymorphisms resulting in increased expression of TLR7 (the ligand for which is single-stranded RNA) are associated with an increased risk of developing SLE[11–13]. TLR7 expression is higher in women than in men owing to the localization of *TLR7* on the X chromosome[14]. One X chromosome is normally inactivated in women; yet, some genes on the X chromosome, including *TLR7*,

Key points

- Intrinsic TLR7 and TLR9 signalling in B cells plays an important role in the development and pathogenesis of systemic lupus erythematosus (SLE).
- In patients with SLE, effector plasma cells are generated via the extrafollicular response and via the formation of spontaneous germinal centres.
- TLR7 plays key roles in the extrafollicular response and the response mediated by germinal centres.
- Some plasma cells produce IL-10 and can have protective roles in lupus-like disease.

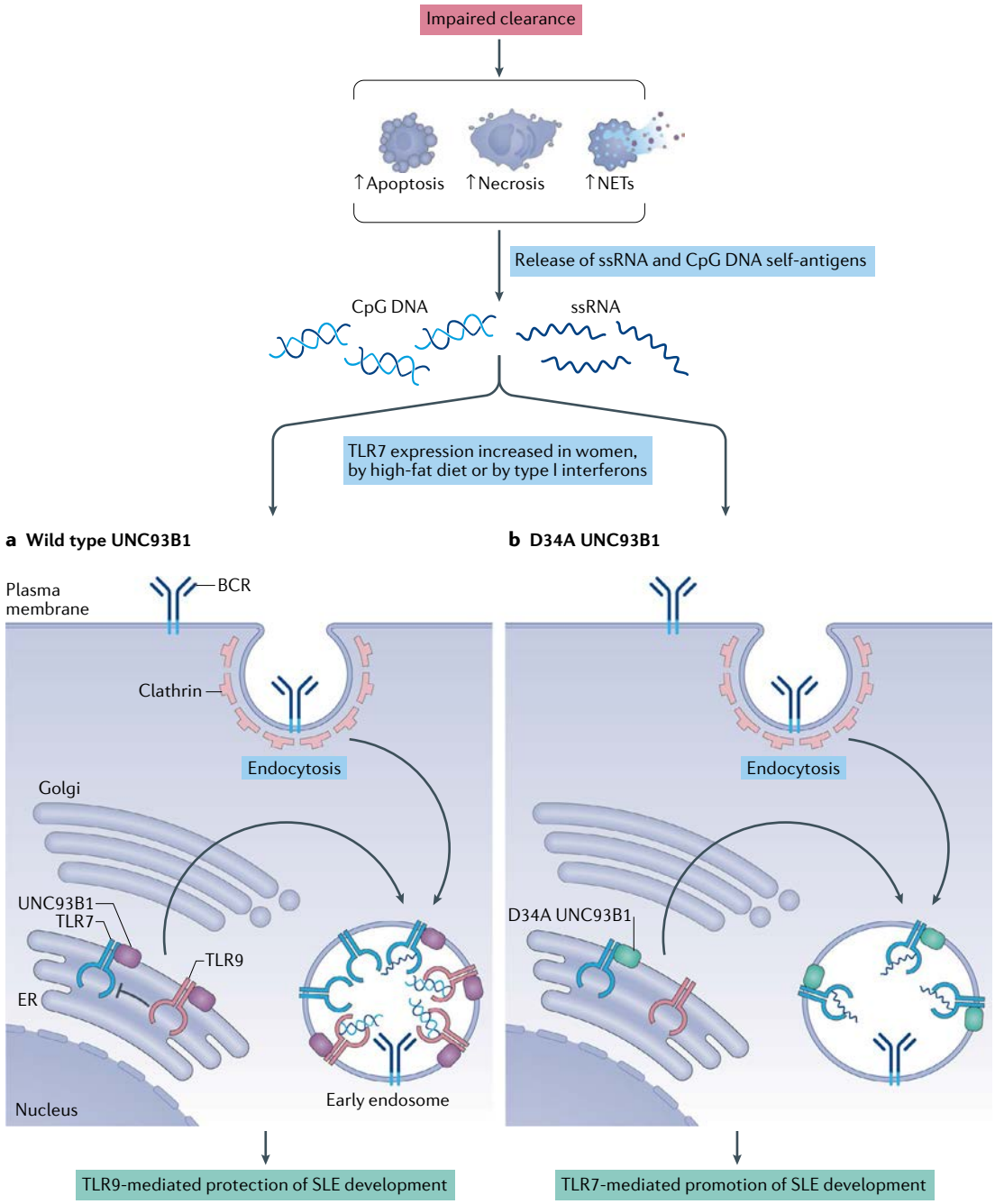
always seem to escape inactivation[14]. As a result, *TLR7* is biallelically expressed in plasmacytoid dendritic cells (pDCs), monocytes and B cells, and TLR7 is thus present at a higher level in these cells in women than in men[14]. In line with this finding, B cells from women exposed to TLR7 agonist in vitro differentiate more efficiently into $CD27^{hi}$ plasmablasts than B cells from men; this gender difference is not observed upon the addition of agonists of TLR9, the gene encoding which is on chromosome 3 (REF.[14]). This observation is consistent with a higher prevalence of SLE in women than in men[15]. Further documenting how X chromosome number affects susceptibility to SLE, the presence of two X chromosomes in men with Klinefelter's syndrome is associated with a higher predisposition to SLE than in men with a single X chromosome[16], and women with a single X chromosome (for example, those with Turner syndrome) are less prone to SLE than women with two X chromosomes[15]. A reduction in TLR7 activity might thus reduce the development of SLE. TLR7 expression is also modulated by metabolic parameters (for example, it is increased by a high-fat diet, which exacerbates SLE)[17], and by cytokines such as type I interferons, which augment the expression of TLR7 but not TLR9 in pDCs[18]. It might thus also be possible to reduce the symptoms of SLE by modulating TLR7 function.

TLR7 predisposes mice to lupus-like disease. TLR7 expression similarly modulates predisposition to lupus-like disease in mice. Overexpression of TLR7 induces systemic autoimmunity in mouse strains not prone to lupus[10,19,20], and the deletion of *Tlr7* reduces lupus development in strains that spontaneously develop such diseases[21,22]. Genetic analysis of the cell types implicated in this reduction underlined the importance of intrinsic TLR7 signalling in B cells in the pathogenesis of lupus-like disease in mice[23]. Specifically, mice that are genetically predisposed to lupus-like disease but have a B cell-specific *Tlr7* deletion displayed reduced disease, lower levels of autoantibodies against RNA-associated and apoptosis-related autoantigens and diminished immune activity, as indicated by a lower number of germinal centre B cells, T follicular helper (T_{FH}) cells, macrophages and neutrophils, including in kidneys; kidneys in these mice had no sign of glomerulonephritis, in contrast to control mice, which were genetically predisposed to lupus-like disease without deletion of *Tlr7* (REF.[23]).

TLR8 and TLR9 protect mice from lupus-like disease. In addition to TLR7, intracellular nucleic acids are detected by TLR8, which also senses single-stranded RNA, and by TLR9, which is a receptor for DNA sequences containing unmethylated cytosine-phosphate-guanosine motifs[24]. Different roles have been identified for these TLRs in distinct models of lupus-like disease. In some models both TLR8 and TLR9 exerted protective effects[25,26]. Specifically, *Tlr8*-null mice and *Tlr9*-null mice displayed more severe lupus than controls, with increased deposition of immunoglobulins and more severe lupus nephritis. These mice also displayed enhanced immune activity and had more germinal centres and antibody-secreting cells, as well as increased autoantibody titres, than controls[26]. Disease exacerbation was abrogated when *Tlr7* was also deleted from *Tlr8*- or *Tlr9*-null mice, indicating that TLR8 and TLR9 might limit the pathogenesis of lupus by limiting the deleterious effects of TLR7 (REF.[26]). Indeed, TLR8 and TLR9 restricted TLR7 activity in dendritic cells and B cells respectively[26,27]. As expected, disease in $Tlr8^{-/-}$ $Tlr9^{-/-}$ double-knockout mice was worse than disease in mice with a single gene defect, reflecting the additive effect of these two abnormalities[26]. Of note, TLR8 does not always act protectively in lupus-like disease in mice because it facilitated the production of anti-RNA antibodies in the absence of *Tlr7* in a model of lupus-like disease in which mice carry a transgenic autoreactive BCR[28]. The cell type responsible for this TLR8-mediated effect was not formally identified in this model, in which TLR7 was the main TLR driving anti-RNA autoantibody production by B cells and TLR9 acted protectively. There is thus no direct evidence that TLR8 signalling can inhibit or increase TLR7 activity in B cells; it might act in other cell types, for instance in neutrophils to increase their secretion of type I interferons[28].

TLR7 and TLR9 functionally interact in B cells. Understanding the functional interaction between TLR7 and TLR9 in B cells relies on understanding how these TLRs are engaged. These TLRs are intracellular and as, unlike dendritic cells, B cells do not internalize extracellular material through micropinocytosis or endocytosis, in B cells they are not directly accessible to natural extracellular nucleic acids[29]. Instead, in B cells, the main portal of antigen entry into cells is through the BCR, which, after engagement, is internalized with the bound antigen and delivered to intracellular compartments, including late endosomes in which TLR7 and TLR9 are present[29–31]. The arrival of BCR–antigen complexes in late endosomes activates these TLRs and triggers the co-stimulation of B cells[29]. This co-stimulation is crucial for autoreactive B cell activation in mouse models of lupus-like disease because deletion of *Tlr7* or *Tlr9* results in the loss of autoantibodies against RNA- or DNA-containing antigens respectively[21,32]. Thus, no pathway appears to be able to compensate for the absence of *Tlr7* or *Tlr9* in the development of lupus-like disease in mice.

Mechanisms of antagonism between TLR7 and TLR9 in B cells. Antagonism between TLR7 and TLR9 can occur within a single B cell if this B cell expresses a BCR that recognizes autoantigens comprising both TLR7 and TLR9 agonists. In this scenario, it was found that TLR9 engagement restrained the differentiation of B cells instructed by TLR7 in vitro[33]. In fact, B cells did



not differentiate into CD138^{hi} antibody-secreting cells unless *Tlr9* was deleted or TLR9 was pharmacologically inhibited upon antigen stimulation[33]. In agreement with this observation, antigens engaging both BCR and TLR7 (but not TLR9) could induce antigen-specific B cell differentiation into CD138^{hi} antibody-secreting cells; this differentiation was not observed for antigens co-engaging the BCR and TLR9, or with synthetic agonists of TLR7 or TLR9 that did not trigger the BCR[33]. Although these in vitro findings do not perfectly recapitulate what is happening in vivo (where the engagement of TLR9 in B cells contributes positively to the production of anti-DNA autoantibodies[27]), they underline functional differences between TLR7 and TLR9 during B cell activation, and the unique response induced upon co-engagement of BCR and TLR7 that is likely relevant to the development of lupus in mice. In keeping with a role for TLR7 in the development of lupus, TLR7 signalling (but not TLR9, TLR2, TLR3 or TLR4 signalling) is strictly required for the formation of spontaneous germinal centres in vivo in mice[34].

The antagonistic interaction between TLR7 and TLR9 within one B cell is underscored by the competition of these TLRs for the intracellular protein UNC93B1, which promotes their trafficking to endosomal compartments[35,36] (FIG. 1). Different amino acids within UNC93B1 bind to TLR7 and TLR9, and UNC93B1 harbouring a N-terminal D34A mutation interacts normally with TLR9 but more strongly with TLR7 than wild type UNC93B1; enhanced UNC93B1–TLR7 binding

◀ Fig. 1 | **Opposing roles of TLR7 and TLR9 in SLE.** Systemic lupus erythematosus (SLE) is characterized by the presence of autoreactive B cells that recognize DNA-associated antigens (such as unmethylated cytosine-phosphate-guanosine (CpG) motifs) and RNA-associated antigens (such as the single-stranded RNA (ssRNA) small nuclear RNA, U11). These self-antigens are thought to be released owing to dysregulated processes that increase the abundance of neutrophil extracellular traps (NETs), necrotic cells or apoptotic cells. These autoantigens can be recognized at the surface of B cells by the B cell receptor (BCR), which initiates their cellular internalization. Once in endosomes, self-antigens can trigger Toll-like receptor 7 (TLR7) and/or TLR9 in B cells. Genetic or environmental signals leading to the overexpression of TLR7 (such as gender, diet and the cytokine environment, including the level of type I interferons) increase the susceptibility of individuals to SLE. Under physiological conditions, TLR7 signalling is restrained by TLR9, which protects individuals from the development of SLE. By contrast, the disruption of TLR9 function can favour TLR7 signalling and facilitate the development of SLE. Such disruption can involve the intracellular protein UNC93B1, which drives TLR7 and TLR9 trafficking to endosomal compartments (**a**). D34A mutation in transmembrane UNC93B1 favours its interaction with TLR7 (**b**), which can raise the abundance of TLR7 in endosomes, intensify TLR7 signalling and initiate a fatal systemic inflammatory syndrome in an experimental model. ER, endoplasmic reticulum.

increases the export of TLR7 from the endoplasmic reticulum to the endosomal compartment, favouring TLR7 signalling over TLR9 signalling. Mice carrying this mutation in *Unc93b1* develop a fatal systemic inflammatory syndrome[35]. In the endolysosomal compartment, the duration of TLR7 signalling is controlled by the interaction of UNC93B1 with Syntenin-1 (also known as syndecan-binding protein (SDCBP)), which can terminate transmembrane receptor signalling by promoting their transport to intralumenal vesicles of multivesicular bodies[37]. Mice with a mutation in *Unc93b1* that negatively affects the interaction of UNC93B1 with Syntenin-1 develop a systemic inflammatory disease similar to TLR7-overexpressing mice[37]. These findings underscore the importance of intracellular TLR7 trafficking in regulating TLR7 signalling. These processes can be altered via naturally occurring mutations; for instance, dogs with a mutation in the C-terminal domain of *UNC93B1* that reduces the interaction of UNC93B1 with Syntenin-1 spontaneously develop cutaneous lupus[38].

TLR7 and TLR9 signalling in B cells might also exert opposite effects on SLE via cell-extrinsic mechanisms that condition the immune environment. Little is known about such possible cell-extrinsic effects of B cells in SLE, which might involve B cell-mediated production of cytokines (such as IL-10) that can increase protection against autoimmune diseases including SLE (see below).

Understanding TLR autoantigens in SLE. A complete understanding of the role of these TLRs (TLR7, TLR8 and TLR9) in SLE pathogenesis requires the source and biochemical properties of the relevant autoantigens to be elucidated. Extracellular nucleic acids are involved in SLE pathogenesis, and mutations in *DNASE1* (encoding deoxyribonuclease-1) have been associated with the development of SLE in humans and lupus-like disease in mice[39,40]. These self-antigens are thought to be released owing to dysregulated cellular processes resulting in an increase in abundance of neutrophil extracellular traps, necrotic cells or apoptotic cells (FIG. 1). Many intracellular autoantigens are redistributed from intracellular sites to the plasma membrane during apoptosis[41], and the impaired clearance of apoptotic cells has been associated with SLE pathogenesis[42]. The small nuclear RNA U11 is also an endogenous agonist of TLR7 that drives immune pathogenesis[43]. Thus, abundantly available nucleic acids in patients with SLE contribute to active and chronic disease, likely by triggering persistent T cell-dependent and T cell-independent B cell activation. Autoreactive lymphocytes might also be activated through 'epitope mimicry' with the microbiota, as documented for autoreactive T cells targeting the SLE autoantigen Ro60 (a protein element of small cytoplasmic ribonucleoprotein hY-RNA complexes) from the host and microbiota[44]. Furthermore, rearrangements of the heavy-chain variable (VH) gene *VH4-34*, which are preferentially employed in autoimmunity, especially the idiotype 9G4 (a particular group of VH4-34-containing antibodies[45]) in SLE[46], which contributes to the autoantibody repertoire against RNA and dsDNA[47], cross-react with the gut microbiota[48]. Notably, impaired B cell selection of $9G4^+$ B cells has been observed in various autoimmune conditions including SLE[46]. Identifying where, when and how these TLR antigens drive effector B cell differentiation will provide important insights into the pathophysiology of SLE.

Effector B cell subsets driven by TLR7

SLE is associated with alterations in B cell homeostasis. Disease flares are marked by expansions in plasmablasts that are discernible in blood and correlate in magnitude with disease exacerbation[49]. These antibody-secreting cells have a more diverse repertoire of BCRs than antibody-secreting cells generated after vaccination with tetanus or influenza[50] and contain cells of irrelevant antigen specificity, with up to 1% of IgG-antibody-secreting cells producing antibodies against influenza virus or tetanus toxin[50,51]. Antibody-secreting cells in these waves also contain few expanded clones that are autoreactive, indicating the presence of an autoantigen-specific response and of cells activated in a bystander manner[50,51].

Differentiation of antibody-secreting B cells. The trajectory of effector B cell subsets leading to these antibody-secreting cells was reconstructed by combining the phenotyping of the peripheral blood B cell compartment by flow cytometry with analysis of the immunoglobulin gene repertoire. This reconstruction identified activated naive B cells ($CD11c^+IgD^+CD27^-CD21^-MTG^+CD23^-$) and the DN2 subset of IgD^-CD27^-double negative B cells (DN2 B cells; $IgD^-CD27^-CD11c^+$T-$Bet^+CD69^+CD21^-CD24^-CD38^-CXCR5^-FCRL4^-FCRL5^+$)[52], both of which are abundant in patients with SLE but rare in healthy individuals, as having a role in the production of antibody-secreting cells. Activated naive B cells have been considered to be the precursors of DN2 B cells, which are prone to differentiating into antibody-secreting cells[52]. Interestingly, activated naive B cells and DN2 B cells have a similar transcriptional profile, which differs by the expression of only 42 genes[52,53]. The transcriptome of DN2 B cells is consistent with their status as precursors to antibody-secreting cells: they express higher levels of BLIMP-1 and IRF4, master transcription factors of plasmablast and plasma cell differentiation[54], as well as of SLAMF7, which is encoded by an IRF4 target gene and also found at higher levels in plasma

cells than in other B cell subsets[52]. Furthermore, they express less ETS1, a transcription factor that inhibits antibody-secreting cell formation, than other B cell subsets[52]. As expected based on these observations, DN2 B cells rapidly secrete antibodies after polyclonal stimulation, including anti-Smith and anti-ribonucleoprotein (RNP), indicating that they express autoreactive BCRs that recognize RNA-associated autoantigens. Supporting the notion that these cells play a notable role in the production of such antibodies, the frequency of DN2 B cells correlates with the levels of anti-Smith and anti-RNP autoantibodies, the two RNA-associated antigens investigated in this study, in patients with SLE[52]. Furthermore, accumulation of $CD11c^+T\text{-}Bet^+CD21^-CD38^-$ B cells resembling DN2 B cells, which were autoreactive and correlated with clinical manifestations, was similarly reported in a different cohort of patients with SLE[55].

Roles of TLR7 in B cell differentiation. TLR7 can instruct successive steps in the differentiation of resting naive B cells to activated naive B cells, DN2 B cells and, subsequently, to antibody-secreting cells, which defines a pathway of extrafollicular B cell differentiation[52]. TLR7 has a stimulatory activity in all of these effector B cell subsets and can promote the differentiation of DN2 B cells, which are hyperresponsive to TLR7 agonists, into antibody-secreting cells in the presence of IL-21 and IFNγ[52]. By contrast, DN2 B cells are less responsive to CD40 engagement than activated naive B cells[52], indicating a change in the responsiveness of B cells to external stimuli during differentiation, perhaps owing to a progressive reduction in the expression of TRAF5, a factor that is required for CD40 signalling and inhibitory for TLR signalling[56,57].

The role of TLR7 in the differentiation of effector B cells in patients with SLE has been further documented through molecular studies in an in vitro culture system that generates DN2-like B cells with a transcriptome similar to that of DN2 B cells from patients with SLE[58]. This culture system relies on the stimulation of naive human B cells with agonists of TLR7 and BCR as well as IFNγ, IL-2, IL-21 and B cell activating factor, and generates $IgD^-CD27^-CD11c^+T\text{-}Bet^{hi}CD21^-CXCR5^-IRF4^{int}FcRL5^+$ B cells resembling DN2 B cells found in patients with SLE[58]. TLR7 signalling was crucial for the subsequent differentiation of these cells into antibody-secreting cells[58]. The sensitivity of B cells to TLR7 agonists is augmented by IFNγ, which confers them with the capacity to respond productively to amounts of TLR7 agonist that are otherwise insufficient[58]. Of note, the concentration of IFNγ is increased in the serum of some patients with SLE[59,60]. By contrast, B cells from patients with SLE are hyporesponsive to TLR9 agonists[61,62], suggesting that the balance between these two opposing TLR signalling pathways is distorted in patients with SLE.

TLR7 also plays a key role in the differentiation of naive B cells into $CD11c^+T\text{-}Bet^+CD21^-$ B cells (which would comprise activated naive and DN2 B cells in humans) in animal models of lupus, because these cells are absent in mice when *Tlr7* is deleted[63]. Furthermore, the repeated administration of TLR7 agonists (but not of TLR3, TLR4 or TLR9 agonists) promotes the accumulation of these cells in mice via a mechanism involving intrinsic TLR7 signalling in B cells[63]. Remarkably, mice with B cell-specific deletion of *Tlr9* display a higher number of $CD11c^+CD11b^+$ activated B cells than their counterpart with functional *Tlr9*, underlining the correlation between the abundance of these cells and the development of lupus-like disease and providing another example of the opposing roles of TLR7 and TLR9 (REF.[23]). Of note, TLR7 also facilitates the formation of spontaneous germinal centres in mice which, in addition to the extrafollicular plasma cell response involving DN2 B cells, can lead to autoantibody production in patients with SLE[64] (FIG. 2). As in patients with SLE, IFNγ is also important for the pathogenic functions of B cells in animal models of lupus, underlining the conserved role of these pathways in this disease across species[65–68].

In conclusion, and as previously discussed[69]suggests that two pathways of B cell activation lead to the formation of autoreactive antibody-secreting cells in patients with SLE: the extrafollicular response (supported by activated naive B cells and DN2 B cells) and germinal centre reactions[47]. Remarkably, intrinsic TLR7 signalling in B cells has emerged as a key player in both responses (FIG. 2). These findings provide a framework in which to carry out the biochemical analyses of the molecular mechanisms implicated in these cellular processes. Interestingly, these pathways are not uniquely confined to autoimmunity as they have been reported to be active in patients with COVID-19 (REF.[70]).

Mechanisms of TLR signalling in B cells

B cells uniquely respond to TLR agonists. B cells are defined by the expression of a cell surface BCR and, as they also express multiple TLRs, these cells are thus at the intersection of adaptive and innate immunity. Intrinsic innate signalling in B cells is essential for the development of lupus-like disease in mice, as lupus nephritis was absent in mice with a B cell-type specific ablation of *Myd88* (*Myd88* encodes the signalling adaptor protein MyD88, which acts downstream of TLRs and IL-1 receptors)[71–73]. The response of B cells to TLR agonists is unique compared with other cell types of the immune system. B cells proliferate intensively and produce large amounts of IL-10 upon TLR stimulation, a combination not observed in myeloid cells[7]. Underlining the complex role of intrinsic TLR signalling in B cells during disease, B cell-derived IL-10 can be protective in autoimmune diseases[74], including in mouse models of lupus-like disease[75]. Thus, in mice deficient in *Lyn*, which encodes a non-receptor tyrosine-protein kinase that regulates innate and adaptive immune responses, IL-10 production by B cells inhibits the progression of lupus-like disease even when no other cell types can produce this cytokine[75].

Interplay between TLR signalling and BCR signalling. These unique features of TLR-driven cellular activation are related to the distinctive expression of BCR on B cells; B cells in which BCR has been genetically ablated fail to proliferate upon TLR stimulation[76,77]. At the intracellular level, TLR-stimulated B cell

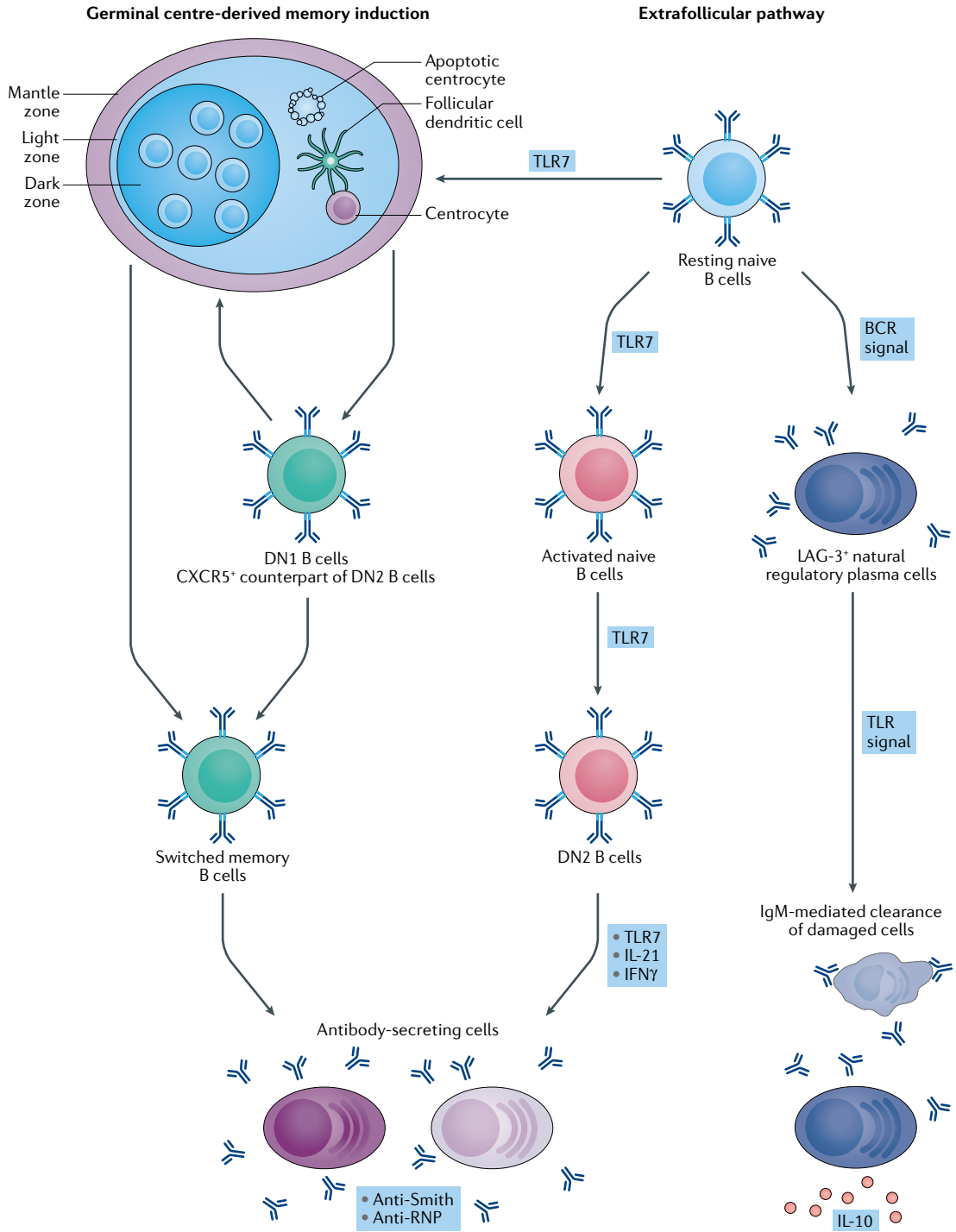


Fig. 2 | **TLRs drive plasma cell differentiation in SLE via different pathways.** Two distinct pathways generate pathogenic antibody-secreting cells in patients with systemic lupus erythematosus (SLE): germinal centre reactions and the extrafollicular pathway, both of which engage resting naive B cells. The germinal centre pathway generates the DN1 subset of double negative B cells (DN1 B cells; that is, IgD^- $CD27^-$ cells that are $CXCR5^+$) and the memory B cells produced in germinal centres can re-enter the germinal centre reaction or differentiate into antibody-secreting cells that produce isotype switched anti-Smith and anti-RNP. The spontaneous generation of the germinal centre is dependent on TLR7. TLR7 also drives the extrafollicular pathway, in which resting naive B cells become activated naive B cells ($CD11c^+IgD^+CD27^-CD21^-MTG^+CD23^-$) and, subsequently, the DN2 subset of IgD^- $CD27^-$ double-negative B cells (DN2 B cells; IgD^- $CD27^-CD11c^+$ $Tbet^+$ $CD69^+CD21^-CD24^-CD38^-CXCR5^-FCRL4^-FCRL5^+$). DN2 B cells are precursors of pathogenic antibody-secreting cells in patients with SLE, the differentiation into which is promoted by TLR7, IL-21 and IFNγ. Of note, resting naive B cells can also generate, in a manner dependent on the B cell receptor (BCR), regulatory plasma cells that are characterized by the cell surface expression of lymphocyte activation gene 3 protein (LAG-3). These regulatory plasma cells produce a uniquely high level of IL-10 in response to TLR signalling. At steady state, they also secrete IgM with reactivity against antigens expressed by damaged cells, suggesting that they might be involved in the clearance of damaged cells.

proliferation involves the adaptor protein Src tyrosine kinase SYK, which promotes BCR signalling by phosphorylating immunoreceptor tyrosine-based activation motifs in the cytoplasmic domains of the Igα and Igβ substructures of the BCR[78,79]. Thus, *Syk*-deficient B cells show impaired proliferation and IL-10 secretion upon activation with TLR agonists[76]. This fact is related to the defective activation of AKT and ERK in the absence of SYK. Remarkably, this TLR–SYK–AKT–ERK pathway is independent of MyD88 as it is still active in *Myd88*-deficient mice[76]. Finally, PI3K also has a key role in this pathway as over-expression of this kinase restored a proliferative response in BCR-deficient B cells stimulated with TLR agonists[77].

The recruitment of the BCR signalling cascade downstream of TLR engagement in B cells underscores the interconnection between the innate and cognate functions of these cells. Along these lines, the engagement of the BCR upregulates TLR expression in human B cells, endowing these cells with the capacity to respond to TLR agonists[5,80]. In this context, genome-wide association studies identified the BCR signalling pathway as the biological process most affected by genetic polymorphisms facilitating the development of SLE[81]. These polymorphisms might not only facilitate BCR signalling but also increase TLR signalling in B cells, which might enable the growth of autoreactive B cell clones and the release of cytokines by them. Interestingly, the two B cell subsets with the highest capacity to produce IL-10 upon TLR stimulation in the mouse, namely LAG-3^{+}CD138^{hi} natural regulatory plasma cells and CD1d^{hi} B cells, develop in a BCR-dependent manner[82,83] (FIG. 2).

TLR signalling also comprises a SYK-independent pathway because the activation of NF-κB and the secretion of IL-6 (events downstream of TLR signalling) occurred normally in *Syk*-deficient B cells stimulated via TLR[76]. IL-6 production by B cells is directly relevant to the pathogenesis of SLE, as IL-6 expression is increased in patients with active SLE[84] and correlates with disease activity in patients with lupus nephritis[85]. IL-6 expression can be induced in B cells by TLR7 agonists[86,87], and this expression is further enhanced by IFNγ[88]. Remarkably, the ablation of IL-6 production specifically from B cells abrogated the spontaneous formation of germinal centres in lupus-prone mice and inhibited disease development[88]. Thus, elimination of the arm of intrinsic TLR signalling in B cells that results in IL-6 production, which seems to be independent of SYK, might be more beneficial in patients with SLE.

From a therapeutic standpoint, the fact that BCR signalling adaptors such as SYK have been implicated in TLR-mediated cell function suggests that inhibitors of BCR signalling might block the TLR-driven functions of B cells. Accordingly, the SYK inhibitor entospletinib reduced human B cell responses to TLR9 agonist[89], and SYK expression was increased in activated CD21^{low} B cells[90].

Targeting TLR signalling to treat SLE

Inhibiting signalling adaptors. There is much interest in inhibiting signalling adaptors implicated in the pro-inflammatory functions of TLRs to treat inflammatory diseases. Although TLRs are prototypic pathogen recognition receptors, humans with loss of function mutations in *MYD88*, which transduces signals via all TLRs except TLR3, display a narrow susceptibility to pyogenic bacterial infections by *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but are resistant to other common microbial pathogens[91]. Remarkably, their susceptibility to these pathogens decreases with age. Similarly, children with a deficiency in *IRAK4* (which encodes IL-1 receptor-associated kinase 4, a component of the TLR signalling pathways important for TLR7 and TLR9, as well as other TLRs) display an increased susceptibility to pyogenic bacterial infections during their first 10 years of life, which improves with age[92]. The improvement of pathogen control in both of these groups of individuals is likely related to the emergence of compensatory adaptive mechanisms involving T lymphocytes or B lymphocytes[93]. The inhibition of key molecules of the TLR signalling pathway, such as MYD88 or IRAK4, might thus allow inflammation to be tapered without compromising host defence against pathogens.

Inhibiting TLR activation in endosomes. The inhibition of endosomal TLR activation appears to be the most pertinent for treating patients with SLE. Existing treatments already target this pathway; notably, hydroxychloroquine and bafilomycin inhibit endosome acidification and/or maturation, thereby inhibiting both TLR7 and TLR9 signalling[94]. Hydroxychloroquine only moderately inhibits TLR signalling, and does not result in a surge of infection, consistent with the concept that TLR inhibition modulates, rather than suppresses, the immune system[95]. It inhibits the inflammatory response of human memory B cells, including their TLR-stimulated production of IL-6 (REF.[96]). Several other inhibitors of endosomal TLRs are under clinical evaluation for use in rheumatic diseases, including SLE and psoriasis, that also involve TLR7 signalling. TLR7, TLR8 and TLR9 signal through IRAK4, the inhibition of which has been studied in various assays and showed superior effects compared with hydroxychloroquine on the inhibition of cytokine production and inflammatory gene expression in peripheral blood mononuclear cells[97]. An early trial using the IRAK4 inhibitor PF06650833 provided promising phase I data in healthy individuals, showing a favourable safety and pharmacokinetic profile as well as evidence of pharmacological effect[98]. Additional inhibitors of IRAK1 and IRAK4 or of TAK1, a signalling molecule involved in TLR signalling, are under development[99]. Of note, these approaches do not differentiate between different endosomal TLRs[95] and they affect multiple cell types in addition to B cells. The safety of these inhibitors thus needs to be considered carefully.

Boosting TLR signalling. Although the dominant rationale for targeting TLRs to treat autoimmune diseases is to inhibit TLR signalling, the data outlined above indicate that some TLR signalling is protective in inflammatory diseases, including in lupus-like disease; thus, boosting TLR signalling to strengthen its regulatory function might be an alternative treatment strategy. Indeed, there is already some insight into the use of TLR agonists in

Table 1 | **TLR modulators in clinical development for inflammatory diseases**

TLR target	Compound	Target disease	Mechanism of action	Development phase (NCT number)	Refs
TLR7	Imiquimod	Actinic keratosis	Immune-stimulator	Phase I (NCT01151956); phase IV (NCT00777127, NCT01453179)	108–112
	GSK2245035	Rhinitis	Induces type I IFN; immune-stimulator	Phase II (NCT01788813, NCT02446613, NCT01607372); phase I (NCT01480271)	113,114
		Asthma	Induces type I IFN; immune-stimulator	Phase II (NCT03707678); phase I/II (NCT02833974)	115
TLR9	CYT003-QbG10	Asthma	Induces a T_H1 cell-mediated immune response	Phase II (NCT02087644, NCT00890734)	116,117
	AZD1419	Asthma	Induces T_H1-type IFN response	Phase II (NCT02898662)	118,119
	Hydroxychloroquine	Sjögren's syndrome	Immune modulator	Phase III (NCT00632866, NCT01601028)	120,121
IRAK4	ND-2158	Rodent models of: lipopolysaccharide-induced TNF production; collagen-induced arthritis; gout; activated B cell like-diffuse large B cell lymphoma; chronic lymphocytic leukaemia	Small molecule inhibitors of inflammatory pathways	Preclinical	122,123
	BMS-986126	Systemic lupus erythematosus	Inhibitor	Preclinical	124
	PF-06650833	Rheumatic autoimmune diseases	Inhibitor	Phase I (NCT02224651, NCT02485769); phase II (NCT02996500)	98
	BAY1834845	Psoriasis; pelvic inflammatory disease	Small-molecule inhibitor	Phase I (NCT03493269, NCT03054402)	125
IRAK1, IRAK4 and TAK1	HS-243	Autoimmune diseases	Inhibitor	Preclinical	99

IRAK-, IL-1 receptor-associated kinase; TAK1, transforming growth factor-β-activated kinase 1; T_H1, T helper 1; TLR, Toll-like receptor.

the clinic, which shows the feasibility and safety of such approaches. Cancer therapy has undertaken approaches to target TLRs with FDA-approved agonists, including: the locally administered Bacillus Calmette–Guérin vaccine (comprising live attenuated *Mycobacterium bovis*) for bladder cancer, which can stimulate TLR2 and TLR4 (microbial cell wall) and TLR9 (bacterial DNA); topical imiquimod for pre-malignant actinic keratosis, which targets TLR7 in basal cell carcinoma; and monophosphoryl lipid A, which is a bioactive part of a lipopolysaccharide targeting TLR4 in human papillomavirus-associated cervical cancer[100]. Further TLR agonists and antagonists are in clinical development for cancer. In this context, TLR agonists are expected to facilitate programmed cell death and enhance immune surveillance[101]. TLR antagonists are used to limit the TLR-driven growth of tumour cells and it should be considered if some of these strategies could be beneficial in autoimmune diseases.

Conclusions

There is increasing evidence that TLR7 has an important role in SLE pathogenesis, with functions conserved in humans and in mice. TLR7 is critical for the extrafollicular and germinal centre responses associated with the activation of autoreactive B cells that is implicated in this disorder. Genetic studies have shown that TLR7 signalling in B cells is particularly important in orchestrating disease. It is remarkable that the different endosomal TLRs that act as nucleic acid sensors, namely TLR7, TLR8 and TLR9, have distinct roles in patients with SLE. In fact, TLR8 and TLR9 might even have beneficial functions in patients with SLE. Uncovering the biochemistry of these molecular processes is thus important and might lead to the identification of novel targets for drug development.

The ability of TLR signalling to activate and inhibit immune signalling, which is not completely understood, suggests that several strategies could target this pathway to treat disease. Although current clinical developments for targeting TLR signalling are still limited compared with treatments for other biological targets[102,103], a number of molecules are currently in development for targeting TLR signalling in inflammatory diseases[104] (TABLE 1). It will be of great interest to follow their clinical development and possible application. Beyond the currently developed approaches, it will also be of interest to identify strategies to rebalance the TLR7 and TLR9 pathways and thus readjust immune homeostasis. It is possible that B cell depletion therapy could reset these pathways by replacing B cells in which this dysregulation might be epigenetically imprinted by novel naive B cells. By contrast, this defect might persist in B cells, including memory B cells that have resisted depletion, thus favouring the restart of disease. Finally, it is important to consider that the inhibition of BCR signalling might interrupt some functions of TLR signalling in B cells[76]. This interruption might be pertinent for the use of the SYK inhibitor fostamatinib, which is approved for treating the autoimmune disease chronic

immune thrombocytopenia, a disease in which the role of intrinsic TLR signalling in B cells is not defined[105].

It remains incompletely understood why TLR7 might be deleterious and TLR9 might be protective in SLE, although it is possible that this phenomenon is related to the distinct types of autoantigens that these TLRs recognize. Some immune complexes containing RNP induced TLR7-mediated production of TNF by macrophages and type I IFN by pDCs[106,107]. However, it is unclear if immune complexes containing TLR9 agonists have similar immunological properties to those containing TLR7 agonists as there has been no systematic comparison of the myeloid cell response to immune complexes associated with RNA (and thus TLR7) versus DNA (and thus TLR9) moieties. There is also some evidence that TLR7 and TLR9 signalling might have opposing roles in SLE given that they distinctively impact B cell activation and differentiation, as mentioned above[33]. However, the relevance of this difference in patients with SLE has not been tested experimentally. Of note, we still have a limited knowledge of the differences in signalling and cellular responses driven by distinct TLRs; these differences might be a fruitful area for future drug development, especially considering that TLR signalling in B cells can have potent anti-inflammatory functions by eliciting the production of IL-10. The distinct molecular mechanisms associated with the control of immunity, including B cell responses, by TLRs thus seems directly relevant for the development of novel therapeutic strategies for autoimmune diseases.

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1. Crickx, E., Weill, J. C., Reynaud, C. A. & Mahevas, M. Anti-CD20-mediated B-cell depletion in autoimmune diseases: successes, failures and future perspectives. *Kidney Int.* **97**, 885–893 (2020).
2. Shen, P. & Fillatreau, S. Antibody-independent functions of B cells: a focus on cytokines. *Nat. Rev. Immunol.* **15**, 441–451 (2015).
3. Davis, M. L. R. et al. Associations of toll-like receptor (TLR)-4 single nucleotide polymorphisms and rheumatoid arthritis disease progression: an observational cohort study. *Int. Immunopharmacol.* **24**, 346–352 (2015).
4. Alzabin, S. et al. Investigation of the role of endosomal toll-like receptors in murine collagen-induced arthritis reveals a potential role for TLR7 in disease maintenance. *Arthritis Res. Ther.* **14**, R142 (2012).
5. Ruprecht, C. R. & Lanzavecchia, A. Toll-like receptor stimulation as a third signal required for activation of human naive B cells. *Eur. J. Immunol.* **36**, 810–816 (2006).
6. Jiang, W. et al. TLR9 stimulation drives naive B cells to proliferate and to attain enhanced antigen presenting function. *Eur. J. Immunol.* **37**, 2205–2213 (2007).
7. Lampropoulou, V. et al. TLR-activated B cells suppress T cell-mediated autoimmunity. *J. Immunol.* **180**, 4763–4773 (2008).
8. Lee, Y. H., Choi, S. J., Ji, J. D. & Song, G. G. Association between toll-like receptor polymorphisms and systemic lupus erythematosus: a meta-analysis update. *Lupus* **25**, 593–601 (2016).
9. Mohan, C. & Putterman, C. Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. *Nat. Rev. Nephrol.* **11**, 329–341 (2015).
10. Pisitkun, P. et al. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. *Science* **312**, 1669–1672 (2006).
11. Garcia-Ortiz, H. et al. Association of TLR7 copy number variation with susceptibility to childhood-onset systemic lupus erythematosus in Mexican population. *Ann. Rheum. Dis.* **69**, 1861–1865 (2010).
12. Conrad, D. F. et al. Origins and functional impact of copy number variation in the human genome. *Nature* **464**, 704–712 (2010).
13. Wang, C. M. et al. Genetic variations in toll-like receptors (TLRs 3/7/8) are associated with systemic lupus erythematosus in a Taiwanese population. *Sci. Rep.* **4**, 3792 (2014).
14. Souyris, M. et al. TLR7 escapes X chromosome inactivation in immune cells. *Sci. Immunol.* **3**, eaap8855 (2018).
This article documents the fact that *TLR7* escapes complete inactivation on the silenced X chromosome in women, leading to a gender difference in its expression that might contribute to the higher incidence of SLE in women than in men.
15. Margery-Muir, A. A., Bundell, C., Nelson, D., Groth, D. M. & Wetherall, J. D. Gender balance in patients with systemic lupus erythematosus. *Autoimmun. Rev.* **16**, 258–268 (2017).
16. Scofield, R. H. et al. Klinefelter's syndrome (47,XXY) in male systemic lupus erythematosus patients: support for the notion of a gene-dose effect from the X chromosome. *Arthritis Rheum.* **58**, 2511–2517 (2008).
17. Hanna Kazazian, N. et al. Lupus autoimmunity and metabolic parameters are exacerbated upon high fat diet-induced obesity due to TLR7 signaling. *Front. Immunol.* **10**, 2015 (2019).
18. Bekeredjian-Ding, I. B. et al. Plasmacytoid dendritic cells control TLR7 sensitivity of naive B cells via type I IFN. *J. Immunol.* **174**, 4043–4050 (2005).
19. Deane, J. A. et al. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. *Immunity* **27**, 801–810 (2007).
20. Walsh, E. R. et al. Dual signaling by innate and adaptive immune receptors is required for TLR7-induced B-cell-mediated autoimmunity. *Proc. Natl Acad. Sci. USA* **109**, 16276–16281 (2012).
21. Christensen, S. R. et al. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. *Immunity* **25**, 417–428 (2006).
22. Fairhurst, A. M. et al. Yaa autoimmune phenotypes are conferred by overexpression of TLR7. *Eur. J. Immunol.* **38**, 1971–1978 (2008).
23. Jackson, S. W. et al. Opposing impact of B cell-intrinsic TLR7 and TLR9 signals on autoantibody repertoire and systemic inflammation. *J. Immunol.* **192**, 4525–4532 (2014).
24. Miyake, K. Nucleic acid-sensing toll-like receptors: beyond ligand search. *Adv. Drug Deliv. Rev.* **60**, 782–785 (2008).
25. Tran, N. L., Manzin-Lorenzi, C. & Santiago-Raber, M. L. Toll-like receptor 8 deletion accelerates autoimmunity in a mouse model of lupus through a toll-like receptor 7-dependent mechanism. *Immunology* **145**, 60–70 (2015).
26. Desnues, B. et al. TLR8 on dendritic cells and TLR9 on B cells restrain TLR7-mediated spontaneous autoimmunity in C57BL/6 mice. *Proc. Natl Acad. Sci. USA* **111**, 1497–1502 (2014).
27. Tilstra, J. S. et al. B cell-intrinsic TLR9 expression is protective in murine lupus. *J. Clin. Invest.* **130**, 3172–3187 (2020).
28. Umiker, B. R. et al. Dosage of X-linked toll-like receptor 8 determines gender differences in the development of systemic lupus erythematosus. *Eur. J. Immunol.* **44**, 1503–1516 (2014).
29. Eckl-Dorna, J. & Batista, F. D. BCR-mediated uptake of antigen linked to TLR9 ligand stimulates B-cell proliferation and antigen-specific plasma cell formation. *Blood* **113**, 3969–3977 (2009).
30. Viglianti, G. A. et al. Activation of autoreactive B cells by CpG dsDNA. *Immunity* **19**, 837–847 (2003).
31. Lau, C. M. et al. RNA-associated autoantigens activate B cells by combined B cell antigen receptor/Toll-like receptor 7 engagement. *J. Exp. Med.* **202**, 1171–1177 (2005).
32. Christensen, S. R. et al. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. *J. Exp. Med.* **202**, 321–331 (2005).
33. Nundel, K. et al. Cell-intrinsic expression of TLR9 in autoreactive B cells constrains BCR/TLR7-dependent responses. *J. Immunol.* **194**, 2504–2512 (2015).
34. Soni, C. et al. B cell-intrinsic TLR7 signaling is essential for the development of spontaneous germinal centers. *J. Immunol.* **193**, 4400–4414 (2014).
35. Fukui, R. et al. Unc93B1 restricts systemic lethal inflammation by orchestrating toll-like receptor 7 and 9 trafficking. *Immunity* **35**, 69–81 (2011).
36. Fukui, R. et al. Unc93B1 biases toll-like receptor responses to nucleic acid in dendritic cells toward DNA- but against RNA-sensing. *J. Exp. Med.* **206**, 1339–1350 (2009).
37. Majer, O., Liu, B., Kreuk, L. S. M., Krogan, N. & Barton, G. M. UNC93B1 recruits syntenin-1 to dampen TLR7 signalling and prevent autoimmunity. *Nature* **575**, 366–370 (2019).
This article documents a mechanism implicated in the termination of TLR7 signalling, and its importance for avoiding systemic autoimmunity.
38. Leeb, T. et al. A missense variant affecting the C-terminal tail of UNC93B1 in dogs with exfoliative cutaneous lupus erythematosus (ECLE). *Genes* **11**, 159 (2020).
39. Yasutomo, K. et al. Mutation of DNASE1 in people with systemic lupus erythematosus. *Nat. Genet.* **28**, 313–314 (2001).
40. Napirei, M. et al. Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nat. Genet.* **25**, 177–181 (2000).
41. Casciola-Rosen, L. A., Anhalt, G. & Rosen, A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**, 1317–1330 (1994).
42. Shao, W. H. & Cohen, P. L. Disturbances of apoptotic cell clearance in systemic lupus erythematosus. *Arthritis Res. Ther.* **13**, 202 (2011).
43. Negishi, H. et al. Identification of U11snRNA as an endogenous agonist of TLR7-mediated immune pathogenesis. *Proc. Natl Acad. Sci. USA* **116**, 23653–23661 (2019).
44. Greiling, T. M. et al. Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus. *Sci. Transl. Med.* **10**, eaan2306 (2018).
This work highlights the potential role of immune crossrecognition of self- and commensal bacterial-expressed Ro60 in triggering SLE.
45. Richardson, C. et al. Molecular basis of 9G4 B cell autoreactivity in human systemic lupus erythematosus. *J. Immunol.* **191**, 4926–4939 (2013).
46. Richardson, C. T. et al. Failure of B Cell Tolerance in CVID. *Front. Immunol.* **10**, 2881 (2019).
47. Jenks, S. A. et al. Distinct effector B cells induced by unregulated toll-like receptor 7 contribute to pathogenic responses in systemic lupus erythematosus. *Immunity* **52**, 203 (2020).
48. Schickel, J. N. et al. Self-reactive VH4-34-expressing IgG B cells recognize commensal bacteria. *J. Exp. Med.* **214**, 1991–2003 (2017).
49. Jacobi, A. M. et al. Correlation between circulating CD27high plasma cells and disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum.* **48**, 1332–1342 (2003).
50. Tipton, C. M. et al. Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. *Nat. Immunol.* **16**, 755–765 (2015).
51. Klinman, D. M. & Steinberg, A. D. Systemic autoimmune disease arises from polyclonal B cell activation. *J. Exp. Med.* **165**, 1755–1760 (1987).

52. Jenks, S. A. et al. Distinct effector B cells induced by unregulated toll-like receptor 7 contribute to pathogenic responses in systemic lupus erythematosus. *Immunity* **49**, 725–739 e726 (2018).
This publication implies a role for TLR7 in the differentiation of effector B cell subsets implicated in SLE.
53. Jenks, S. A., Cashman, K. S., Woodruff, M. C., Lee, F. E. & Sanz, I. Extrafollicular responses in humans and SLE. *Immunol. Rev.* **288**, 136–148 (2019).
54. Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M. & Corcoran, L. M. The generation of antibody-secreting plasma cells. *Nat. Rev. Immunol.* **15**, 160–171 (2015).
55. Wang, S. et al. IL-21 drives expansion and plasma cell differentiation of autoreactive CD11c^{hi}T-bet^{+} B cells in SLE. *Nat. Commun.* **9**, 1758 (2018).
56. Buchta, C. M. & Bishop, G. A. TRAF5 negatively regulates TLR signaling in B lymphocytes. *J. Immunol.* **192**, 145–150 (2014).
57. Kraus, Z. J., Nakano, H. & Bishop, G. A. TRAF5 is a critical mediator of in vitro signals and in vivo functions of LMP1, the viral oncogenic mimic of CD40. *Proc. Natl Acad. Sci. USA* **106**, 17140–17145 (2009).
58. Zumaquero, E. et al. IFNγ induces epigenetic programming of human T-bet^{hi} B cells and promotes TLR7/8 and IL-21 induced differentiation. *eLife* **8**, e41641 (2019).
59. Lu, R. et al. Dysregulation of innate and adaptive serum mediators precedes systemic lupus erythematosus classification and improves prognostic accuracy of autoantibodies. *J. Autoimmun.* **74**, 182–193 (2016).
60. Munroe, M. E. et al. Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Ann. Rheum. Dis.* **75**, 2014–2021 (2016).
61. Gies, V. et al. Impaired TLR9 responses in B cells from patients with systemic lupus erythematosus. *JCI Insight* **3**, e96795 (2018).
62. Sieber, J. et al. Active systemic lupus erythematosus is associated with a reduced cytokine production by B cells in response to TLR9 stimulation. *Arthritis Res. Ther.* **16**, 477 (2014).
63. Rubtsov, A. V. et al. Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c^{+} B-cell population is important for the development of autoimmunity. *Blood* **118**, 1305–1315 (2011).
64. Boneparth, A. et al. TLR7 influences germinal center selection in murine SLE. *PLoS One* **10**, e0119925 (2015).
65. Domeier, P. P. et al. IFN-γ receptor and STAT1 signaling in B cells are central to spontaneous germinal center formation and autoimmunity. *J. Exp. Med.* **213**, 715–732 (2016).
66. Jackson, S. W. et al. B cell IFN-gamma receptor signaling promotes autoimmune germinal centers via cell-intrinsic induction of BCL-6. *J. Exp. Med.* **213**, 733–750 (2016).
67. Thibault, D. L. et al. IRF9 and STAT1 are required for IgG autoantibody production and B cell expression of TLR7 in mice. *J. Clin. Invest.* **118**, 1417–1426 (2008).
68. Chodisetti, S. B. et al. Type II but not type I IFN signaling is indispensable for TLR7-promoted development of autoreactive B cells and systemic autoimmunity. *J. Immunol.* **204**, 796–809 (2020).
69. Dorner, T. & Lipsky, P. E. B cells: depletion or functional modulation in rheumatic diseases. *Curr. Opin. Rheumatol.* **26**, 228–236 (2014).
70. Woodruff, M. et al. Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19. *Nat. Immunol.* **21**, 1506–1516 (2020).
71. Becker-Herman, S. et al. WASp-deficient B cells play a critical, cell-intrinsic role in triggering autoimmunity. *J. Exp. Med.* **208**, 2033–2042 (2011).
72. Teichmann, L. L., Schenten, D., Medzhitov, R., Kashgarian, M. & Shlomchik, M. J. Signals via the adaptor MyD88 in B cells and DCs make distinct and synergistic contributions to immune activation and tissue damage in lupus. *Immunity* **38**, 528–540 (2013).
73. Hua, Z. et al. Requirement for MyD88 signaling in B cells and dendritic cells for germinal center anti-nuclear antibody production in Lyn-deficient mice. *J. Immunol.* **192**, 875–885 (2014).
74. Fillatreau, S., Sweenie, C. H., McGeachy, M. J., Gray, D. & Anderton, S. M. B cells regulate autoimmunity by provision of IL-10. *Nat. Immunol.* **3**, 944–950 (2002).
75. Scapini, P. et al. B cell-derived IL-10 suppresses inflammatory disease in Lyn-deficient mice. *Proc. Natl Acad. Sci. USA* **108**, E823–E832 (2011).
76. Schweighoffer, E., Nys, J., Vanes, L., Smithers, N. & Tybulewicz, V. L. J. TLR4 signals in B lymphocytes are transduced via the B cell antigen receptor and SYK. *J. Exp. Med.* **214**, 1269–1280 (2017).
This article implies a role for the BCR and its key signalling adaptor SYK in TLR signalling in B cells.
77. Otipoby, K. L. et al. The B-cell antigen receptor integrates adaptive and innate immune signals. *Proc. Natl Acad. Sci. USA* **112**, 12145–12150 (2015).
78. Reth, M. & Brummer, T. Feedback regulation of lymphocyte signalling. *Nat. Rev. Immunol.* **4**, 269–277 (2004).
79. Ackermann, J. A. et al. Syk tyrosine kinase is critical for B cell antibody responses and memory B cell survival. *J. Immunol.* **194**, 4650–4656 (2015).
80. Bernasconi, N. L., Onai, N. & Lanzavecchia, A. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. *Blood* **101**, 4500–4504 (2003).
81. Julia, A. et al. Genome-wide association study meta-analysis identifies five new loci for systemic lupus erythematosus. *Arthritis Res. Ther.* **20**, 100 (2018).
82. Lino, A. C. et al. LAG-3 inhibitory receptor expression identifies immunosuppressive natural regulatory plasma cells. *Immunity* **49**, 120–133 e129 (2018).
This article reports the identification of a subset of natural regulatory plasma cells suppressing immunity in an IL-10-dependent manner and under the command of TLR signalling.
83. Yanaba, K., Bouaziz, J. D., Matsushita, T., Tsubata, T. & Tedder, T. F. The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. *J. Immunol.* **182**, 7459–7472 (2009).
84. Tackey, E., Lipsky, P. E. & Illei, G. G. Rationale for interleukin-6 blockade in systemic lupus erythematosus. *Lupus* **13**, 339–343 (2004).
85. Abdel Galil, S. M., Ezzeldin, N. & El-Boshy, M. E. The role of serum IL-17 and IL-6 as biomarkers of disease activity and predictors of remission in patients with lupus nephritis. *Cytokine* **76**, 280–287 (2015).
86. Glaum, M. C. et al. Toll-like receptor 7-induced naive human B-cell differentiation and immunoglobulin production. *J. Allergy Clin. Immunol.* **123**, 224–230 e224 (2009).
87. Hanten, J. A. et al. Comparison of human B cell activation by TLR7 and TLR9 agonists. *BMC Immunol.* **9**, 39 (2008).
88. Arkatkar, T. et al. B cell-derived IL-6 initiates spontaneous germinal center formation during systemic autoimmunity. *J. Exp. Med.* **214**, 3207–3217 (2017).
This article demonstrates that the formation of spontaneous germinal centres playing a key role in humoral immunity is controlled by the production of IL-6 by B cells, linking their antibody-dependent and antibody-independent functions.
89. Weissenberg, S. Y. et al. Identification and characterization of post-activated B cells in systemic autoimmune diseases. *Front. Immunol.* **10**, 2136 (2019).
90. Keller, B. et al. High SYK expression drives constitutive activation of CD21^{low} B cells. *J. Immunol.* **198**, 4285–4292 (2017).
91. von Bernuth, H. et al. Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* **321**, 691–696 (2008).
92. Ku, C. L. et al. Selective predisposition to bacterial infections in IRAK-4-deficient children: IRAK-4-dependent TLRs are otherwise redundant in protective immunity. *J. Exp. Med.* **204**, 2407–2422 (2007).
93. Slack, E. et al. Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* **325**, 617–620 (2009).
94. Ahmad-Nejad, P. et al. Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. *Eur. J. Immunol.* **32**, 1958–1968 (2002).
95. Schrezenmeier, E. & Dorner, T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat. Rev. Rheumatol.* **16**, 155–166 (2020).
96. Torigoe, M. et al. Hydroxychloroquine efficiently suppresses inflammatory responses of human class-switched memory B cells via toll-like receptor 9 inhibition. *Clin. Immunol.* **195**, 1–7 (2018).
97. Hjorton, K. et al. Cytokine production by activated plasmacytoid dendritic cells and natural killer cells is suppressed by an IRAK4 inhibitor. *Arthritis Res. Ther.* **20**, 238 (2018).
98. Danto, S. I. et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of PF-06650833, a selective interleukin-1 receptor-associated kinase 4 (IRAK4) inhibitor, in single and multiple ascending dose randomized phase 1 studies in healthy subjects. *Arthritis Res. Ther.* **21**, 269 (2019).
99. Scarneo, S. A. et al. A highly selective inhibitor of interleukin-1 receptor-associated kinases 1/4 (IRAK-1/4) delineates the distinct signaling roles of IRAK-1/4 and the TAK1 kinase. *J. Biol. Chem.* **295**, 1565–1574 (2020).
100. Braunstein, M. J., Kucharczyk, J. & Adams, S. Targeting Toll-like receptors for cancer therapy. *Target. Oncol.* **13**, 583–598 (2018).
101. Cen, X., Liu, S. & Cheng, K. The role of Toll-like receptor in inflammation and tumor immunity. *Front. Pharmacol.* **9**, 878 (2018).
102. Felten, R. et al. The 2018 pipeline of targeted therapies under clinical development for systemic lupus erythematosus: a systematic review of trials. *Autoimmun. Rev.* **17**, 781–790 (2018).
103. Dorner, T. & Furie, R. Novel paradigms in systemic lupus erythematosus. *Lancet* **393**, 2344–2358 (2019).
104. Javaid, N., Yasmeen, F. & Choi, S. Toll-like receptors and relevant emerging therapeutics with reference to delivery methods. *Pharmaceutics* **11**, 441 (2019).
105. Roskoski, R. Jr. Properties of FDA-approved small molecule protein kinase inhibitors: a 2020 update. *Pharmacol. Res.* **152**, 104609 (2020).
106. Berggren, O. et al. B lymphocytes enhance interferon-alpha production by plasmacytoid dendritic cells. *Arthritis Rheum.* **64**, 3409–3419 (2012).
107. Mold, C. & Clos, T. W. C-reactive protein inhibits plasmacytoid dendritic cell interferon responses to autoantibody immune complexes. *Arthritis Rheum.* **65**, 1891–1901 (2013).
108. Goldenberg, G., Linkner, R. V., Singer, G. & Frankel, A. An investigator-initiated study to assess the safety and efficacy of imiquimod 3.75% cream when used after cryotherapy in the treatment of hypertrophic actinic keratoses on dorsal hands and forearms. *J. Clin. Aesthet. Dermatol.* **6**, 36–43 (2013).
109. Hadley, J. et al. Results of an investigator-initiated single-blind split-face comparison of photodynamic therapy and 5% imiquimod cream for the treatment of actinic keratoses. *Dermatol. Surg.* **38**, 722–727 (2012).
110. Serra-Guillen, C. et al. A randomized pilot comparative study of topical methyl aminolevulinate photodynamic therapy versus imiquimod 5% versus sequential application of both therapies in immunocompetent patients with actinic keratosis: clinical and histologic outcomes. *J. Am. Acad. Dermatol.* **66**, e131–e137 (2012).
111. Strohal, R., Kerl, H. & Schuster, L. Treatment of actinic keratoses with 5% topical imiquimod: a multicenter prospective observational study from 93 Austrian office-based dermatologists. *J. Drugs Dermatol.* **11**, 574–578 (2012).
112. Gollnick, H., Dirschka, T., Ostendorf, R., Kerl, H. & Kunstfeld, R. Long-term clinical outcomes of imiquimod 5% cream vs. diclofenac 3% gel for actinic keratosis on the face or scalp: a pooled analysis of two randomized controlled trials. *J. Eur. Acad. Dermatol. Venereol.* **34**, 82–89 (2020).
113. Ellis, A. K., Tsitoura, D. C., Quint, D., Powley, W. & Lee, L. A. Safety and pharmacodynamics of intranasal GSK2245035, a TLR7 agonist for allergic rhinitis: a randomized trial. *Clin. Exp. Allergy* **47**, 1193–1203 (2017).
114. Tsitoura, D. et al. Early clinical evaluation of the intranasal TLR7 agonist GSK2245035: use of translational biomarkers to guide dosing and confirm target engagement. *Clin. Pharmacol. Ther.* **98**, 369–380 (2015).
115. Biggadike, K. et al. Discovery of 6-Amino-2-{[(1 S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-pu rin-8-one (GSK2245035), a highly potent and selective intranasal toll-like receptor 7 agonist for the treatment of asthma. *J. Med. Chem.* **59**, 1711–1726 (2016).
116. Casale, T. B. et al. CYT003, a TLR9 agonist, in persistent allergic asthma - a randomized placebo-controlled Phase 2b study. *Allergy* **70**, 1160–1168 (2015).
117. Beeh, K. M. et al. The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. *J. Allergy Clin. Immunol.* **131**, 866–874 (2013).
118. Jackson, S. et al. First-in-human study with the inhaled TLR9 oligonucleotide agonist AZD1419 results in interferon responses in the lung, and is safe and

well-tolerated. *Clin. Pharmacol. Ther.* **104**, 335–345 (2018).
119. Psallidas, I. et al. A phase 2a, double-blind, Placebo-controlled randomized trial of inhaled TLR9 agonist AZD1419 in asthma. *Am. J. Respir. Crit. Care Med.* https://doi.org/10.1164/rccm.202001-0133OC (2020).
120. Gottenberg, J. E. et al. Effects of hydroxychloroquine on symptomatic improvement in primary Sjogren syndrome: the JOQUER randomized clinical trial. *JAMA* **312**, 249–258 (2014).
121. Yoon, C. H. et al. Effect of hydroxychloroquine treatment on dry eyes in subjects with primary Sjogren's syndrome: a double-blind randomized control study. *J. Korean Med. Sci.* **31**, 1127–1135 (2016).
122. Kelly, P. N. et al. Selective interleukin-1 receptor-associated kinase 4 inhibitors for the treatment of autoimmune disorders and lymphoid malignancy. *J. Exp. Med.* **212**, 2189–2201 (2015).
123. Gimenez, N. et al. Targeting IRAK4 disrupts inflammatory pathways and delays tumor development in chronic lymphocytic leukemia. *Leukemia* **34**, 100–114 (2020).
124. Dudhgaonkar, S. et al. Selective IRAK4 inhibition attenuates disease in murine lupus models and demonstrates steroid sparing activity. *J. Immunol.* **198**, 1308–1319 (2017).
125. Wiese, M. D., Manning-Bennett, A. T. & Abuhelwa, A. Y. Investigational IRAK-4 inhibitors for the treatment of rheumatoid arthritis. *Expert Opin. Investig. Drugs* **29**, 475–482 (2020).

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Axial spondyloarthritis: concept, construct, classification and implications for therapy

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Abstract | The axial spondyloarthritis (axSpA) disease concept has undergone substantial change from when the entity ankylosing spondylitis was defined by the modified New York criteria in 1984. Developments in imaging, therapy and genetics have all contributed to changing the concept of axSpA from one of erosions in the sacroiliac joints to a spectrum of disease with and without changes evident on plain radiographs. Changes to the previously held concept and construct of the disease have also necessitated new classification criteria. The use of MRI, primarily of the sacroiliac joints, has substantially altered the diagnosis and differential diagnosis of axSpA. Many in the axSpA community believe that the current classification criteria lack specificity, and the CLASSIC study is underway to examine this area. Although much about the evolving axSpA disease concept is universally agreed, there remains disagreement about operationalizing aspects of it, such as the requirement for the objective demonstration of axial inflammation for the classification of axSpA. New imaging technologies, biomarkers and genetics data will probably necessitate ongoing revision of axSpA classification criteria. Advances in our knowledge of the biology of axSpA will settle some differences in opinion as to how the disease concept is applied to the classification and diagnosis of patients.

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Axial spondyloarthritis (axSpA) is a disease commonly encountered in the field of rheumatology. The concept of axSpA — that is, the idea of what it is (BOX 1) — is the result of the recognition of the early phases of the disease historically termed ankylosing spondylitis (AS). Historically, in the era of the modified New York criteria, sacroiliac damage had to be evident on plain radiographs to fulfil the criteria for AS[1]. MRI, however, has since opened our eyes to an expanded disease spectrum. The recognition that inflammation is present in the spine and sacroiliac joints prior to the development of erosions revealed an earlier phase of axSpA in an objective way that was not possible previously. The construct of the disease, a type of operational definition, has thus had to evolve to include this expanded spectrum, as illustrated in FIG. 1. This new construct in turn led to the development of new classification criteria for axSpA to enable investigation of this neglected early part of the disease spectrum in a reproducible way. The process of constructing classification criteria highlighted the differing views of academics and clinicians around the world about what constitutes the construct of axSpA.

Despite some disagreement, there is much about the axSpA construct that is universally accepted. First, the concept of non-radiographic axSpA (nr-axSpA) has been introduced to complement the widely known and recognized disease entity AS. axSpA is now recognized as an umbrella term that encompasses both AS and nr-axSpA and a continuum from early or mild to late or severe disease. Information from imaging studies, primarily using MRI, is helping to characterize the range of changes seen in the axial skeleton in health and disease, as only in understanding the full spectrum of manifestations can we understand where 'normal' stops and 'disease' starts. In addition, in many areas in science and medicine we learn so much about the fundamentals of a phenomenon when the ability to influence it becomes available, as with new therapies. In this way, how axSpA responds to treatment and what this response means about its underlying biology has given fruitful insights into what the disease is and what it is not. This Review examines the theoretical basis for axSpA, the concept and the construct, and the resultant classification criteria that have been proposed. In addition, we discuss some of the issues identified in diagnosing and classifying axSpA, and further explore the nature of axSpA in the context of theories such as the philosophical concept of 'natural kinds' and latent class analysis of SpA. Fundamentally redefining a disease is

Key points

- The concept of axial spondyloarthritis (axSpA) has expanded from ankylosing spondylitis with evidence of erosions to a spectrum of disease encompassing non-radiographic axSpA and radiographic axSpA.
- The current classification criteria capture the entire spectrum of axSpA, but many in the field believe they lack specificity; the CLASSIC study is underway to further assess this issue.
- The concept of axSpA is largely agreed upon in the research community, but opinion still diverges about some aspects, for example, the demonstration of objective axial inflammation for axSpA classification.
- The current definition of a positive sacroiliac joint MRI scan lacks specificity for axSpA, as demonstrated in imaging studies of individuals with and without back pain and post-partum women.
- Concepts such as the theory of natural kinds and latent class analysis enable us to further examine the crucial features of the axSpA concept, with sacroiliitis being the core feature.
- Advances in our understanding of the biology of axSpA via novel imaging, genetic and biomarker studies will probably enable the resolution of many current issues in axSpA diagnosis and classification.

not a common occurrence, and in this Review we also explore how this considerable change has affected the recognition of axSpA in the clinic, the use of imaging and access to therapies. We conclude by discussing unanswered questions and potentially fruitful areas of further research.

Spondyloarthritis and axSpA

The SpA umbrella. Any discussion of axSpA must start with reviewing the overarching concept of spondyloarthritis (SpA) as a whole. SpA as a disease label has been applied to presentations of disease that include a wide range of individual elements[2] (FIG. 2). These elements include spinal inflammatory disease, with the pathological feature being a polyenthesitis of the vertebral column. In addition, peripheral inflammatory arthritis, peripheral enthesitis (for example, Achilles tendonitis), dactylitis, anterior uveitis, skin psoriasis, non-specific urethritis, conjunctivitis, aortitis and inflammatory bowel disease (IBD) are also recognizable elements that make up the constellation of features recognized as SpA. These features often, but not always, manifest in loosely defined groups, which historically have then attracted their own (sub)labels, including AS, psoriatic arthritis (PsA), enteropathic arthritis (IBD-associated arthritis) and reactive arthritis (formally referred to as Reiter syndrome[3]). The term 'undifferentiated SpA' has also been used when the constellation of features is more recognizable as SpA than as another entity, such as rheumatoid arthritis, but does not fall within one of the loosely defined (sub)groups, such as PsA[4,5]. At least initially, Behçet disease was also included in the schema of SpA owing to descriptions of polyarthritis, sacroiliitis and seronegativity in series of patients with Behçet disease[6]; however, this classification has not been widely adopted and genetic association studies have failed to find important shared genetic factors[6–8]. Historically, vital motivation for devising the SpA concept was to distinguish PsA from rheumatoid arthritis with coincident psoriasis by the absence of rheumatoid factor, which generated the terms 'seronegative arthritis' and 'seronegative SpA'[9].

However, one of the challenges of this historical (sub)grouping approach has been that patients with similar clinical features can fall into different groups because of a marked overlap of clinical features between the entities. However, some distinct clinical presentations exist that support a 'splitting' approach[10,11]. For example, dactylitis is more closely associated with PsA than with other forms of SpA, and urethritis and conjunctivitis are more closely associated with reactive arthritis. The problems inherent in lumping all spondyloarthropathies together as SpA or splitting SpA into subgroups such as PsA and AS has led to a movement to instead describe (or at least classify) SpA as either axSpA or peripheral SpA (pSpA). This phenotypic approach has some appeal because similar presentations attract the same label. For instance, PsA and enteropathic arthritis, which both manifest as lower limb inflammatory oligoarthritis, would both be referred to as peripheral SpA. As another example, AS and PsA with predominantly axial involvement would both be referred to as axSpA. However, this schema does not address the issue of which descriptor to use when patients have features that fit with both. Therefore, axSpA sits as a phenotypic description of a member of the SpA group with axial involvement. The best way in which to subdivide SpA remains unclear; some aspects of subdivision have demonstrable value but myriad issues arise when subdivision is attempted.

The concept and construct of axSpA. As explained in BOX 1, generally speaking, concepts are ideas that may or may not be solely theoretical, whereas constructs are built-up, operational structures that apply to real instances. The concept of axSpA is universally accepted in rheumatology, as evidenced by its inclusion in academic papers, textbooks and as a topic at clinical and academic meetings[12,13]. Also, doctors seeing patients with the constellation of symptoms that has come to be

Box 1 | **axSpA disease construct, concept, classification and diagnosis**[28,30,90,91]

Concept: a concept is an abstract idea, and can extend to include both known examples and unknown examples. The idea may or may not refer to something that exists in the real world. An example of a concept in the field of axial spondyloarthritis (axSpA) might be the presence of axSpA in the absence of objective signs such as elevated C-reactive protein or sacroiliac joint inflammation on MRI; this concept is an abstract idea that may or may not exist.

Construct: a construct is an abstract idea that contains conceptual elements. Constructs are more specific and less abstract than concepts. Constructs encompass actual cases, whereas concepts extend over both actual and possible cases. axSpA is itself a construct, which includes conceptual elements such as sacroiliac joint inflammation, spinal inflammation and associated features such as anterior uveitis.

Classification criteria: classification criteria provide a standardized definition of a disease to enable the identification of a homogeneous group of cases for research purposes. A set of classification criteria does not capture the whole spectrum of manifestations of a disease, but should be highly specific in order to minimize false-positive errors. An example in axSpA is the 2009 Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axSpA[16].

Diagnostic criteria: diagnostic criteria are a set of signs, symptoms and tests for use in ordinary clinical practice to guide the care of individual patients. They should have near perfect positive and negative predictive value (which is rare). No diagnostic criteria exist for axSpA or SpA.

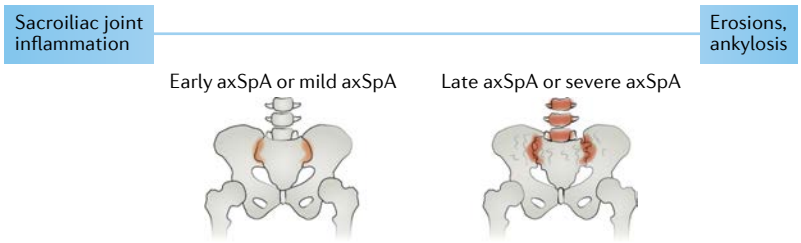

Fig. 1 | **The spectrum of axial spondyloarthritis.** In this illustration, the spectrum of axial spondyloarthritis (axSpA) is shown extending from early (or mild) disease, involving only inflammation in the sacroiliac joints, through to severe (or late) disease with erosive damage in the sacroiliac joints. This schematic is not meant to imply that early and mild disease, or late and severe disease, are synonymous, only that a similar spectrum concept exists.

known as axSpA is a universal experience. Although this manuscript is concerned with the concept and construct of axSpA, from an operational point of view axSpA is viewed as an inflammatory disorder of the axial spine and sacroiliac joints, and nr-axSpA is a subcategory of this disorder, in which plain radiographs of the pelvis do not show radiographic damage that meets the modified New York criteria for AS (grade 2 bilateral or grade 3 unilateral damage), whereas in radiographic axSpA (r-axSpA, or AS) these criteria are met[1].

There is also universal acceptance about the clinical features that constitute the construct (FIG. 2). These elements make up SpA as a whole, and the specific label axSpA is applied when axial involvement is predominantly present. From empirical evidence, however, when these disease elements are applied by clinicians in different settings to reach a diagnosis of axSpA, the resultant patient cohort is remarkably variable demographically, genetically and clinically (see the section below on classification criteria). This variability suggests that the elements of the construct are assigned different relative values by different clinicians when deciding on a diagnosis.

Diagnosis of axSpA

A diagnosis of axSpA is generally considered in the presence of chronic back pain with onset before the age of 45 years, although onset of axSpA after this age has also been described[14,15]. The back pain can be 'inflammatory' in nature (inflammatory back pain) but this is not a rule; in typical axSpA cohorts, 63–92% of patients have inflammatory back pain according to various classification criteria[16,17]. If features typically associated with axSpA are present (FIG. 2), imaging with plain radiography of the pelvis is commonly undertaken and if unequivocal radiographic sacroiliitis is apparent, then often the diagnosis of axSpA is made at this point. If the plain radiograph is normal or equivocal, as it often is because the changes are not advanced or bowel or soft tissue is overlying, then MRI of the sacroiliac joints is often ordered. In cases of axSpA, sacroiliac joint MRI often reveals bone marrow oedema and/or fatty lesions and sometimes structural changes such as erosions. The use of gadolinium contrast agent can also enable visualization of synovitis, capsulitis and enthesitis, although the additive value of using contrast-enhanced MRI for the diagnosis of axSpA has been shown to be negligible[18,19].

Research into the value of sacroiliac joint MRI for the diagnosis of axSpA has yielded a wide range of MRI sensitivity[20]. The only study to use a non-clinician diagnostic standard involved analysis of biopsy-obtained sacroiliac joint tissue, and in this study sacroiliac joint MRI was found to have a sensitivity of 38%[21]. However, sacroiliac joint biopsy has not been extensively studied and is not used clinically in the diagnosis of axSpA; thus, the value of this approach as the gold standard of underlying diagnosis is very uncertain. Lacking a positive sacroiliac joint on MRI the diagnosis might also be made, at least provisionally, on the basis of an elevated C-reactive protein (CRP) concentration (in the absence of any other explanation for this elevation). This point does, however, promote robust debate in the axSpA community. MRI can be repeated with the aim of demonstrating objective inflammatory sacroiliitis, as an elevated CRP concentration lacks specificity in this context[22,23]. However, it should be noted that the value of repeat MRI is largely limited to use in those who are male and/or HLA-B27 positive[22,24,25]. A CRP test can also be repeated following an initial normal result, as 'CRP positivity' varies over time in those with nr-axSpA and it is not uncommon for some individuals with AS to have universally normal CRP concentrations[26]. In the clinical diagnostic process, differential diagnoses are considered (TABLE 1) and alternative explanations for abnormal findings are also considered. For example, an elevated CRP concentration can be found in obese but otherwise healthy patients, and can also arise from other diseases, such as IBD[27].

Once a diagnosis is assigned, the diagnostic label is allocated. The field of rheumatology is currently in transition from using the labels 'AS' and 'nr-axSpA' to using the overall label axSpA with the sub-labels 'r-axSpA' and 'nr-axSpA'. The term AS is losing relevance as the emphasis is now shifting to considering axSpA as a continuum from non-radiographic to radiographic disease (FIG. 3). Notably, this whole label transition now underway is based on the long-held erroneous belief that a diagnosis of AS requires radiographic sacroiliitis. This was not the intention of the modified New York criteria for AS[1], which were called 'diagnostic criteria' but were intended to be applied to groups of patients rather than individuals (BOX 1). The science of criteria construction has developed considerably since the publication of the modified New York criteria in the 1980s. At that time, criteria intended for epidemiological studies such as surveys and prevalence estimates were called diagnostic criteria[1], whereas diagnostic criteria are now constructed for use in individual patients and classification criteria are constructed for groups of patients with a disease[28–30].

Imaging and the axSpA construct

A detailed discussion of imaging in axSpA is outside the scope of this article; an excellent contemporary review on the subject is available elsewhere[31]. The influence of imaging on the concept of axSpA has been to demonstrate the presence of inflammation in the absence of radiographically evident disease. This advance in imaging was arguably the stimulus to re-examine the concept of axSpA, which, as mentioned above, previously required radiographic evidence of damage in the

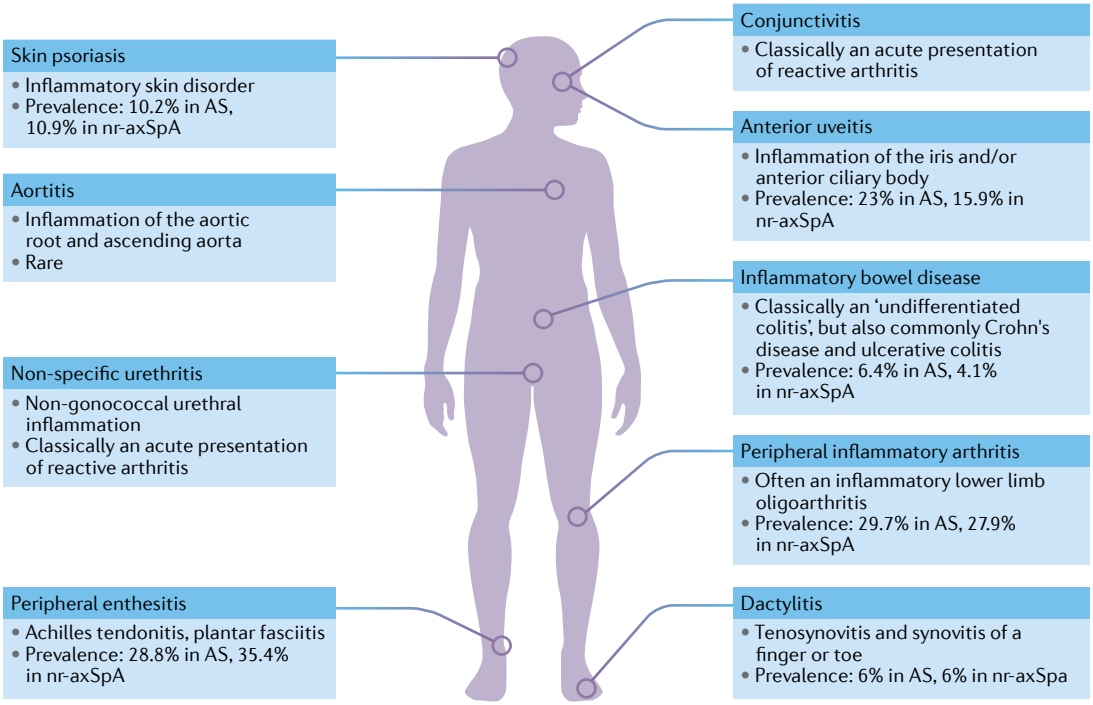

Fig. 2 | **Clinical features of axial spondyloarthritis in addition to axial disease.** The extra-axial features of axial spondyloarthritis (axSpA) are shown. Prevalence rates in ankylosing spondyloarthritis (AS) and non-radiographic axial spondyloarthritis (nr-axSpA) are from de Winter et al.[2].

sacroiliac joints. MRI has consequently improved the confidence of physicians in assigning a diagnosis of axSpA to patients who formerly in clinical practice would have had no objective signs of axial inflammation. This former lack of an objective test for sacroiliac joint inflammation probably contributed to long diagnostic delays in patients with axSpA[32], which were also caused by excessive weight being assigned to the absence of radiographic sacroiliitis and by difficulty with the interpretation of plain radiographs of the sacroiliac joints. The application of MRI has now highlighted that little weight should be given to negative plain radiography findings.

The use of MRI has enabled us to move to a state where axSpA can be more confidently identified at an earlier stage than when sacroiliac joint erosions is demonstrated on plain radiographs. This state, however, has introduced additional issues to be addressed. Significant and/or severe axSpA does not present a diagnostic conundrum; however, the use of sensitive imaging techniques has presented challenges such as identifying where normal variation stops and disease starts.

There remains a high degree of uncertainty about the implications of 'abnormal' findings on MRI. The issue now is to differentiate early or mild disease from normal variation in the population. Erosions on plain radiographs are highly specific for AS (or r-axSpA, to use the emerging terminology), but when this highly specific feature is not required for diagnosis or classification (because the concept of axSpA is now one of axial inflammation and does not require axial joint damage) then diagnostic certainty is reduced. This reduction in certainty is because the symptom of inflammatory back pain lacks specificity, inflammatory markers can commonly be normal and rates of 'abnormal' sacroiliac joint MRI scans are high in non-axSpA populations both with and without back pain[17,26,33–38] (TABLE 2).

The changing classification of axSpA

Historical and current classification. Classification criteria are a research tool that should promote homogeneity among groups of patients and should be applied to patients in whom a clinical diagnosis has already been made[30]. Criteria for classification should have a high specificity (>90%) in order to avoid misclassification (that is, the inclusion of patients who do not have the disease).

As the concept of AS–axSpA has changed considerably over the past few decades, so have the proposed classification criteria[1,16,39,40] (FIG. 4). Radiographic sacroiliitis has long been regarded as the hallmark of the disease, and was required to fulfil either the original or the modified New York criteria, thus reflecting the prevailing view of AS as a disease that causes radiographic damage evident on plain radiographs[1,16,39] (FIG. 4). In retrospect, the new, broader concept of axSpA first emerged in 1985. In a study of first-degree relatives of HLA-B27-positive patients with AS, the presence of "spondylitic disease without radiologic evidence of sacroiliitis" was reported in some of these first-degree relatives, many of whom were female[41]. Despite having some of the clinical features of SpA, these relatives did not fulfil the modified New York criteria[1].

The new concept is that only a proportion of patients with nr-axSpA will progress to r-axSpA (AS); the rest will continue to have nr-axSpA or the disease will spontaneously resolve (FIG. 3). Rates of progression from nr-axSpA to r-axSpA (AS) have been reported in

Table 1 | **Differential diagnosis of axial spondyloarthritis**

Diagnosis	Descriptor
Non-specific low back pain	Chronic back pain with normal or abnormal imaging
Diffuse idiopathic skeletal hyperostosis	Ligamentous calcifications and/or ossifications around the spine
Fracture	Fracture of the vertebral body, spinous process or transverse process and osteoporotic stress fracture
Degenerative arthritis	Back pain, and abnormal spinal imaging
Septic arthritis of the sacroiliac joint and/or spine	Back pain, elevated inflammatory markers and/or abnormal imaging
Crystal arthritis	Inflammatory crystal arthritis that can affect the spinal column
Osteitis condensans ilii	Back pain and abnormal sacroiliac joint imaging post-pregnancy

different cohorts as 1–12% over 2 years, 6–46% over 2–9 years and 26–59% over >10 years[42–51].

In recognition of the broadening concept of axSpA, the 2009 Assessment of SpondyloArthritis International Society (ASAS) classification criteria were developed for the full spectrum of axSpA[16,30]. However, these criteria are not sufficiently specific[16,52,53]: their sensitivity and specificity were reported as 82.9% and 84.4%, respectively[16,52]. Which features help to explain the low specificity of the current ASAS classification criteria for axSpA? It can be concluded that the lack of specificity of these criteria reflects the way they were derived. Briefly, the criteria were derived first by experts assessing 71 'paper patients' (theoretical case vignettes), most of which lacked radiographic sacroiliitis. An additional 649 cases were contributed by ASAS members from 25 centres in 16 countries; these patients had to have had back pain for >3 months with an onset prior to the age of 45. In their routine clinical work-up, 391 (60%) of the 649 patients were diagnosed with axSpA. Among these 391 patients, 52% were male, 66% were HLA-B27 positive, 62% had a normal CRP concentration and 30% met the modified New York criteria for AS. Of the remaining non-axSpA patients, 28% were HLA-B27 positive, three times the background population prevalence of HLA-B27 in white populations. The variance between contributing centres in HLA-B27 was not reported in these papers.

Of note, a subsequent study of genetic profiling[54] in a subset of the patient cohort used in developing the ASAS axSpA classification criteria provides some clues to understanding the low (84.4%) specificity of those criteria[16,52,54]. In this study, the patients, who were from nine centres in six countries, were classified according to the ASAS criteria for axSpA and, using the clinical data supplied, further classified according to the modified New York criteria for AS[1,54]. The results indicate that different centres had very different views on how to arrive at a clinical diagnosis, as evidenced by the differing prevalence of HLA-B27 between the centres (even within the same country), which might reflect either issues with recruiting into the cohort or differences between physicians in what they consider the axSpA construct to be. For example, the HLA-B27 prevalence ranged from 21% to 70%, the proportion of female patients from 5% to 71%, the proportion of patients with axSpA was between 37% and 90%, and the proportion of patients meeting the modified New York criteria ranged from 0% to 48%[54].

In the presence of a gold standard for a disease (for example, in gout, the clear demonstration of urate crystals in a sterile inflamed joint) one might expect a correct diagnosis in all cases, and classification criteria for that disease would have 100% specificity. However, the situation is quite different for a disease such as axSpA, with a broader concept of disease that newly includes the notion of non-radiographic disease, a condition for which there is no gold standard. In this context, the diagnosis can only be based on expert opinion, taking into consideration a plethora of clinical signs and symptoms, a few highly non-specific biomarkers (HLA-B27 and CRP concentration) and imaging results (MRI)[37]. Therefore, it seems very probable that the demonstrated heterogeneity in establishing a clinical diagnosis of axSpA on the basis of experts' opinions is responsible for the subsequent substantial lack of specificity of the ASAS classification criteria for axSpA.

Fundamental to some of the disagreements in the axSpA community is the idea that axSpA can be classified in the absence of objective signs of inflammation. Therefore, it is important to address the issues around the lack of specificity of sacroiliac joint MRI findings. Thus, for some the axSpA construct requires objective inflammation (and therefore it is definitely required for classification) and for others it does not.

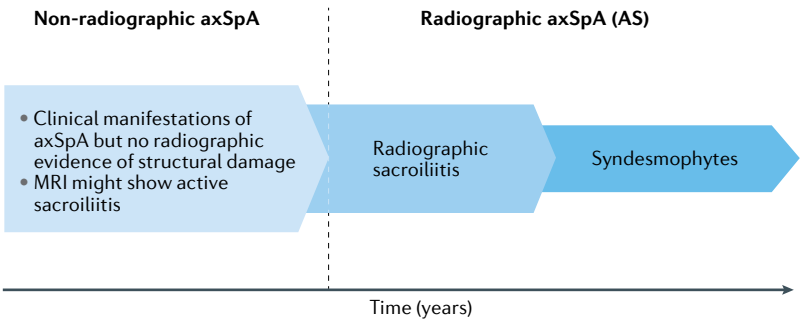

Fig. 3 | **The concept of axial spondyloarthritis.** The concept of axial spondyloarthritis (axSpA) now encompasses non-radiographic (nr-axSpA) and radiographic axSpA (or ankylosing spondylitis (AS)). The arbitrary division between these two entities is becoming less relevant clinically. The decreasing sizes of the three chevrons emphasizes that a decreasing proportion of patients progress to each subsequent stage. In other words, only some patients with nr-axSpA will develop radiographic axSpA (AS), whereas others might continue to have nr-axSpA, perhaps forever, or have a self-limiting disease course. This figure also illustrates that not all patients with radiographic sacroiliitis progress to form syndesmophytes and consequently spinal ankylosis. Adapted with permission from Rudwaleit et al.[30], Wiley.

Diseases as natural kinds. Thinking about SpA as a natural kind (BOX 2) might help to clarify the distinctions between the disorders included under the SpA umbrella and how to identify the cluster of properties that are characteristic of each disease kind. For example, radiographic ankylosis of the sacroiliac joints is a pathognomonic feature of AS, yet many people with AS do not and never will exhibit this degree of sacroiliitis and others who are diagnosed in the early stage of disease

do not manifest any degree of radiographic sacroiliitis[50]. Similarly, HLA-B27 is found in more people without SpA than with SpA but HLA-B27 positivity is considered to be a disposition towards certain modes of antigen presentation that can manifest in AS[8]. The precise label (AS or axSpA) is less important than the concept of the disease as a kind. On the other hand, the disposition to sacroiliac joint ankylosis is not especially necessary or sufficient for PsA, whereas the disposition towards psoriasis is much more important. The recognition that it is possible to fulfil the CASPAR classification criteria for PsA without actually having manifest psoriasis underscores the recognition that it is the disposition towards psoriasis, rather than its manifestation, that is most salient[55].

Latent class analysis. Latent class analysis is a modelling methodology that can be used to classify items. The basic tenet of this methodology is that unobserved (latent) categories (classes) in a system or model differ by observable characteristics[56]. Class membership can be estimated using assumptions of independence of the observable variables. The latent class analysis of patients with SpA within the DEvenir des Spondylarthropathies Indifférenciées Récentes (DESIR) and the SpondyloArthritis Caught Early (SPACE) cohorts provides some support for a dispositional perspective[57]. These cohorts include people with inflammatory back pain and clinically diagnosed axSpA (DESIR) and those with chronic low back pain with onset before age 45 years (SPACE). Latent class analysis of the SPACE cohort identified four clusters of individuals: those with axial disease, which was most strongly associated with imaging evidence of sacroiliitis and HLA-B27; those at risk of disease, which was most strongly associated with a family history

Table 2 | **Studies reporting positive MRI scans in populations with and without axSpA**

Study population	*n*	Sex	Back pain	Proportion with a positive MRI scan[a]	Study	Ref.
Healthy men	29	Male	No	0%	Seven et al. (2019)	33
Hospital cleaning staff	26	Female	No	4%	Seven et al. (2019)	33
Long-distance runners	23	Male and female	No	4%	Seven et al. (2019)	33
Individuals with chronic back pain	47	Male and female	Yes	6%	De Winter et al. (2018)	34
Individuals with lumbar disc herniation	25	Male and female	Yes	8%	Seven et al. (2019)	33
Runners	24	Male and female	No	13%	De Winter et al. (2019)	34
Participants in a community health study	793	Male and female	57%[b]	17%	Baraliakos et al. (2019)	35
Women without post-partum buttock and/or pelvic pain	14	Female	No	21%	Seven et al. (2019)	33
Individuals with chronic back pain	1,020	Male and female	Yes	21%	Arnbak et al. (2016)	89
Healthy individuals	47	Male and female	No	23%	De Winter et al. (2018)	34
Runners (post-running)	20	Male and female	NS	30%	Weber et al. (2018)	37
Runners (pre-running)	20	Male and female	NS	35%	Weber et al. (2018)	37
Military recruits (at baseline)	11	Male and female	No	41%	Varkas et al. (2018)	36
Women with post-partum buttock and/or pelvic pain	46	Female	Yes	41%	Seven et al. (2019)	33
Elite ice hockey players	22	Male	NS	41%	Weber et al. (2018)	37
Military recruits after 6 weeks' training	11	Male and female	No	50%	Varkas et al. (2018)	36
Individuals with axSpA	41	Male and female	Yes	56%	Seven et al. (2019)	33
Women with post-partum back pain	7	Female	Yes	57%	De Winter et al. (2018)	34
Post-partum women within 10 days of vaginal delivery	25	Female	31%	64%	Renson et al. (2020)	38
Individuals with axSpA	47	Male and female	Yes	92%	De Winter et al. (2018)	34

axSpA, axial spondyloarthritis; NRS, numeric rating scale; NS, not specified; SpA, spondyloarthritis. [a]According to the current Assessment of SpondyloArthritis International Society definition of a positive sacroiliac joint MRI for the classification of SpA[20]. [b]On a 0–10 NRS for back pain, of 0, 28% NRS between 1 and 3, and 29% NRS ≥4.

Rome criteria for AS (1961) (ref.[40])

Clinical criteria:

1. Low back pain and stiffness of >3 months' duration that is not relieved by rest.
2. Pain and stiffness in the thoracic region.
3. Limited motion in the lumbar spine.
4. Limited chest expansion.
5. History or evidence of iritis or its sequelae.

Radiological criterion:

6. X-ray showing bilateral sacroiliac changes characteristic of AS (this would exclude bilateral osteoarthrosis of sacroiliac joints).

Definite diagnosis of AS:
- Four of the five clinical criteria are fulfilled; or
- Radiological criterion plus one other criterion are fulfilled.

New York criteria for AS (1968) (ref.[39])

Clinical criteria:

1. Limitation of motion of the lumbar spine in all 3 planes (anterior flexion, lateral flexion, and extension)
2. A history of pain or the presence of pain at the dorsolumbar junction or in the lumbar spine.
3. Limitation of chest expansion to 1 inch (2.5cm) or less, measured at the level of the fourth intercostal space.

Definite AS:
- Grade 3–4 bilateral sacroiliitis associated with at least 1 clinical criterion; or
- Grade 3–4 unilateral or grade 2 bilateral sacroiliitis associated with clinical criterion 1 or with both clinical criteria 2 and 3.

Probable AS:
- Grade 3–4 bilateral sacroiliitis without any signs or symptoms satisfying the clinical criteria.

Modified New York criteria for AS (1984) (ref.[1])

Clinical criteria:

1. Low back pain and stiffness for more than 3 months, which improves with exercise but is not relieved by rest.
2. Limitation of motion of the lumbar spine in both the sagittal and frontal planes.
3. Limitation of chest expansion relative to normal values corrected for age and sex.

Radiological criterion:
- Grade 2–4 bilateral or grade 3–4 unilateral sacroiliitis.

Definite AS:
- Radiological criterion fulfilled in association with at least one clinical criterion.

Probable AS:
- Three clinical criteria are fulfilled; or
- Radiological criterion is fulfilled without any signs or symptoms satisfying the clinical criteria. (Other causes of sacroiliitis should be considered.)

ASAS criteria for axSpA (2009) (ref.[16])

Entry criterion:
- Back pain of ≥3 months' duration and age at onset <45 years.

Classification of axSpA by imaging arm:
- Sacroiliitis on imaging and at least one SpA feature.

Classification of axSpA by HLA-B27 arm:
- HLA-B27-positive plus two or more SpA features.

SpA features:
- Inflammatory back pain, arthritis, heel enthesitis, uveitis, dactylitis, psoriasis, Crohn's disease or ulcerative colitis, good response to NSAIDs, family history of SpA, HLA-B27, elevated CRP concentration.

Sacroiliitis on imaging:
- Active (acute) inflammation on MRI highly suggestive of sacroiliitis associated with SpA; or
- Definite radiographic sacroiliitis as per the modified New York criteria.

Fig. 4 | **Classification criteria for axSpA.** Proposed classification criteria for ankylosing spondylitis (AS) and axial spondyloarthritis (axSpA) have changed considerably over the past few decades, reflecting changes in the concept of the disease. ASAS, Assessment of SpondyloArthritis International Society; CRP, C-reactive protein.

of SpA and HLA-B27; those with no SpA; and those with back pain as well as peripheral arthritis and/or enthesitis. Furthermore, these phenotypes tended to remain stable over time in the DESIR cohort[57].

For the disease entity of AS–axSpA, it seems that the disposition towards sacroiliitis is crucial to the concept of this disease. Sacroiliitis is central to the modified New York criteria[1]. For the 2009 ASAS axSpA classification criteria, features contributing to the classification of axSpA included radiographic sacroiliitis, which had an odds ratio (OR) of 32.3, and active inflammation of sacroiliac joints on MRI, which had an OR of 66.7; by comparison, all other features had ORs of approximately 1–7 (REF.[16]). These ORs also suggest that clinicians feel that sacroiliitis is the crucial feature of the concept of axSpA.

Towards improving axSpA classification. A way forward for improving the specificity of classification criteria to approach 100% would be to develop new classification criteria, beginning with a thorough discussion among their developers with the aim of building consensus about the diagnosis using a wide variety of real patients. One should define the construct of the disease: which clinical and biologic dispositions characterize the disease to be classified and which features would be aberrant? This approach seems particularly important to consider in the context of a new, broadened disease concept in the absence of a gold standard. The consensus about the disease construct should of course be properly assessed by thorough appraisal of observer variation. Other issues to be addressed to improve specificity include the need for standardized diagnostic work-up and comparability across referral patterns, to avoid or reduce diagnostic bias.

One issue that has generated considerable debate is that, at its core, axSpA is defined by axial inflammation, more specifically sacroiliac joint inflammation. However, the ASAS 2009 criteria enable classification of axSpA without objective evidence of axial inflammation. For the purposes of diagnosis and the inherent pragmatism that it requires, this issue is not so important. However, to be true to the aim of classification criteria — that is, to assemble a homogeneous group of patients for clinical study — classification of axSpA without objective evidence of inflammation moves away from the accepted core concept of the disease. Newer imaging techniques and/or biomarkers might enable us to demonstrate sacroiliac joint inflammation in different ways, but at present the absence of objective evidence is a challenge to the long-held concept of axial inflammation.

The basic framework for classification criteria in rheumatology, which has generally been followed since the development of the 2010 ACR–EULAR rheumatoid arthritis criteria, consists of the following elements: a statement regarding to whom the criteria should be applied; specification of the elements or items of the criteria; determination of the relative weight of the individual elements, and a statement of how the elements of the criteria should be combined to arrive at a (usually) binary result (that is, the presence or absence of the health condition of interest); and determination of the accuracy of all the criteria. Overall accuracy is generally expressed as the proportion of people who have the health condition of interest who are also deemed to have the condition according to the criteria (sensitivity), and as the proportion of people do not have the health condition who are also deemed to not to have the condition

Box 2 | **Natural kinds**

In philosophy, 'natural kinds' refer to the idea that some objects can be classified and resemble each other in important ways[92]. The classical example of natural kinds in physical sciences are the chemical elements in the periodic table: each element is a natural kind. An understanding of what it is to be a natural kind might help with disease nosology and conceptualization of disease kinds, if it were the case that diseases are in fact natural kinds. Although that claim is not altogether settled, it is still potentially useful to consider spondyloarthritis conditions through this lens.

One important concept of natural kinds is the homeostatic property cluster (HPC) theory of kinds, which roughly holds that kind membership is about sharing a cluster of properties and that causal forces exist that explain the co-instantiation of these property clusters[93]. When applying the HPC concept of natural kinds to disease, a useful extension is to consider a key property of the kind to be a disposition, rather than a manifestation. For example, radiographic juxta-articular erosions are a characteristic manifestation of rheumatoid arthritis, but not all patients with rheumatoid arthritis exhibit this manifestation, especially in early-stage disease. The disposition towards erosive disease can be considered one of the cluster of properties that characterize rheumatoid arthritis. Similarly, acute anterior uveitis is characteristic of SpA diseases, but only occurs in a minority of cases[94]. Thus, a disposition towards developing acute anterior uveitis is a member of the HPC.

according to the criteria (specificity). Some of these elements can be derived from empirical data, but some rely greatly on expert knowledge and opinion.

To independently validate the 2009 ASAS criteria, the Classification of Axial SpondyloarthritiS Inception Cohort (CLASSIC) study has been established[58]. This multinational study will largely replicate the methods of the original ASAS classification study[16]. The CLASSIC study investigators aim to recruit 1,000 consecutive patients referred to a rheumatologist because of back pain for >3 months and who are <45 years of age[58]. If the specificity of the ASAS criteria is ≥90% and the sensitivity ≥75%, no further investigation of the criteria will reportedly be undertaken; however, if the criteria do not meet these thresholds then refinements will reportedly be made and tested[59]. The current ASAS criteria for axSpA were not derived using relative weighting of each element of the disease, and this technique might be one to consider to better align the construct of axSpA with the resultant classification criteria.

Therapy and the axSpA construct

In trying to clarify where normal variation ends and disease begins, the response of symptoms to effective therapies can potentially provide insight. Most patients with non-inflammatory causes of low back pain do not respond well to treatment with TNF inhibitors[60]; thus, it is possible that response to TNF inhibitors could be helpful in distinguishing normal variation on MRI from axSpA. In clinical trials of adalimumab, golimumab and etanercept, patients with axSpA with elevated CRP concentration and/or sacroiliitis on MRI at baseline responded better to treatment than those with a normal CRP concentration and/or no sacroiliitis on MRI[61–64].

Advisory bodies for single-payer systems such as The National Institute for Health and Care Excellence in the UK and the Pharmaceutical Benefits Advisory Committee in Australia have made increasingly rigorous assessments of applications to licence and fund new therapies for axSpA[65,66]. The FDA has also meticulously assessed applications to register biologics for the treatment of nr-axSpA, and after holding public hearings initially elected not to register the TNF inhibitors adalimumab and certolizumab pegol for this indication on the basis of the trial data presented[64,67,68]. Questions raised by the FDA and EMA when examining applications for the registration of biologics for nr-axSpA concerned the natural history of nr-axSpA, the rate of spontaneous remission and the potential for over-treatment with TNF inhibitors[67,69]. Although the response rates in patients without sacroiliitis on MRI and/or normal CRP concentration were lower than in those with objective signs of disease, they were not numerically similar to placebo. Is this observation a demonstration that the axSpA construct should apply in the absence of an elevated CRP concentration or an abnormal MRI? Is this mild disease, early disease or both, and what is the prognosis of this type of disease? These are questions that remain unanswered at present.

Another trial of certolizumab pegol in nr-axSpA has since been performed and in 2019 the FDA approved this agent for use in the treatment of nr-axSpA[70]. The issue around the registration of certolizumab had centred on how trial participants' plain pelvic radiographs were read in the RAPID-axSpA trial[68]. Initially, radiographs were read locally at each centre where patients were enrolled and managed. However, when this procedure was revised and radiographs were read centrally by a small group of expert readers, an appreciable proportion of patients had their films assessed as AS rather than nr-axSpA; as the reported cohort included a proportion of patients with AS[67] the FDA therefore felt that the outcome of the trial could not be relied upon as a good assessment of the efficacy of certolizumab in nr-axSpA. This incident is an example of the limitations of plain radiographs of the sacroiliac joints, the examination of which has very low inter-reader and intra-reader reliability, and which are increasingly seen as having little relevance to the wider concept of axSpA[31]. The reduced therapeutic response to TNF inhibitors in those who lack objective evidence of inflammation led regulators (such as the EMA and FDA) and agencies that make funding recommendations (for example, the Pharmaceutical Benefits Advisory Committee) to stipulate the presence of inflammation as a requirement for treatment with TNF inhibitors for nr-axSpA when the medications were first registered[65].

Consensus, disagreement and questions

We have moved to a new era in which it is broadly agreed that axial inflammation, and specifically sacroiliac joint inflammation, is a core element of the axSpA construct. However, there remains a divergence of opinion concerning individuals who have symptoms that could be attributed to axSpA but who lack MRI or CRP evidence of axial inflammation. That such patients could potentially fulfil the 2009 ASAS classification criteria for nr-axSpA has caused debate in the SpA community, as some do not believe that patients without objective evidence of inflammation should be included in the axSpA construct[53,67,71–74]. Therefore, the 2009 ASAS classification criteria are believed by some to lack specificity[53].

Owing to the absence of explicit diagnostic criteria for axSpA, classification criteria have, at times, been presented as an alternative. However, diagnosis is not their primary purpose. As mentioned above, the sensitivity and specificity of the 2009 ASAS axSpA criteria are 83% and 84%, respectively, which means that an appreciable proportion of axSpA cases are missed, or individuals without axSpA are included, when classification criteria are used directly in the clinic; classification criteria also do not exclude 'disease mimickers' or 'look-alikes'[30,59,75].

The unanswered questions in the field revolve firstly around the specificity and sensitivity of sacroiliac joint MRI. A growing body of evidence suggests that healthy individuals have a high rate of positive sacroiliac joint MRI (TABLE 2), as it is currently defined by ASAS[20]; in some subgroups, such as post-partum women, this rate can be as high as 64%[38]. Part of the issue could be the lack of familiarity of radiologists with axSpA imaging, or the scanning technique used, but more important is the lack of specificity of the ASAS definition of a positive sacroiliac joint MRI scan[20,76–80]. There is evidence that including structural or erosive change, in addition to evidence of inflammatory activity (bone marrow oedema), increases the specificity of the definition[81,82]. Progress in this area is already being made via proposed changes to imaging protocols[83]. Second, and linked to the first point, is the question of the value (or not) of scanning the spine in addition to the sacroiliac joints. To date, the conclusion has been that there is a limited role for this additional imaging; however, some evidence suggests that a proportion of patients have spine-limited disease that spares the sacroiliac joints[84–86]. Third, what is the natural history of nr-axSpA, including prognostic factors, rates of spontaneous remission and risk factors for progression? Finally, the role of other biomarkers such as genetics and the microbiome[87,88] requires better clarification. Research aimed at addressing these questions could provide clarity on prognosis and identify predictors of response to therapy, as well as potential new therapies.

Conclusions

Owing to advances in imaging techniques, the concept of axSpA has expanded to include axial inflammation that does not (or has not) caused erosive damage. This shift has enabled the recognition and treatment of disease in many people who previously would not have received a diagnosis of axSpA. It has also brought a new set of challenges, primarily distinguishing normal variation from early or mild disease; research to try to clarify this difficult issue is ongoing. The intensity of interest on the part of the public, industry and academia is encouraging, as axSpA has been blighted by long diagnostic delays and a lack of effective treatment since the disease has been recognized. This situation is starting to change, but there is certainly ample scope to improve further for the benefit of our patients.

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1. van der Linden, S., Valkenburg, H. A. & Cats, A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum.* **27**, 361–368 (1984).
2. de Winter, J. J. et al. Prevalence of peripheral and extra-articular disease in ankylosing spondylitis versus non-radiographic axial spondyloarthritis: a meta-analysis. *Arthritis Res. Ther.* **18**, 196 (2016).
3. Panush, R. S., Paraschiv, D. & Dorff, R. E. The tainted legacy of Hans Reiter. *Semin. Arthritis Rheum.* **32**, 231–236 (2003).
4. Dougados, M. et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum.* **34**, 1218–1227 (1991).
5. Zochling, J., Brandt, J. & Braun, J. The current concept of spondyloarthritis with special emphasis on undifferentiated spondyloarthritis. *Rheumatology* **44**, 1483–1491 (2005).
6. Moll, J. M. et al. Associations between ankylosing spondylitis, psoriatic arthritis, Reiter's disease, the intestinal arthropathies, and Behcet's syndrome. *Medicine* **53**, 343–364 (1974).
7. Kirino, Y. et al. Genome-wide association analysis identifies new susceptibility loci for Behcet's disease and epistasis between *HLA-B*51* and *ERAP1*. *Nat. Genet.* **45**, 202–207 (2013).
8. International Genetics of Ankylosing Spondylitis Consortium (IGAS). et al. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat. Genet.* **45**, 730–738 (2013).
9. Wright, V. & Moll, J. The Meaning of Seronegativity and Seropositivity, in Seronegative Polyarthritis. (Elsevier/North-Holland Biomedical Press, 1976).
10. Nash, P. et al. Seronegative spondyloarthropathies: to lump or split? *Ann. Rheum. Dis.* **64**, ii9–ii13 (2005).
11. Taylor, W. J. & Robinson, P. C. Classification criteria: peripheral spondyloarthropathy and psoriatic arthritis. *Curr. Rheumatol. Rep.* **15**, 317 (2013).
12. Sieper, J. & Poddubnyy, D. Axial spondyloarthritis. *Lancet* **390**, 73–84 (2017).
13. Taurog, J. D., Chhabra, A. & Colbert, R. A. Ankylosing spondylitis and axial spondyloarthritis. *N. Engl. J. Med.* **374**, 2563–2574 (2016).
14. Olivieri, I. et al. Spondyloarthritis with onset after age 45. *Curr. Rheumatol. Rep.* **15**, 374 (2013).
15. Toussirot, E. Late-onset ankylosing spondylitis and spondylarthritis: an update on clinical manifestations, differential diagnosis and pharmacological therapies. *Drugs Aging* **27**, 523–531 (2010).
16. Rudwaleit, M. et al. The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann. Rheum. Dis.* **68**, 777–783 (2009).
17. Poddubnyy, D. et al. Diagnostic accuracy of inflammatory back pain for axial spondyloarthritis in rheumatological care. *RMD Open* **4**, e000825 (2018).
18. Hermann, K. G. et al. Magnetic resonance imaging of inflammatory lesions in the spine in ankylosing spondylitis clinical trials: is paramagnetic contrast medium necessary? *J. Rheumatol.* **32**, 2056–2060 (2005).
19. de Hooge, M. et al. Magnetic resonance imaging of the sacroiliac joints in the early detection of spondyloarthritis: no added value of gadolinium compared with short tau inversion recovery sequence. *Rheumatology* **52**, 1220–1224 (2013).
20. Maksymowych, W. P. et al. MRI lesions in the sacroiliac joints of patients with spondyloarthritis: an update of definitions and validation by the ASAS MRI working group. *Ann. Rheum. Dis.* **78**, 1550–1558 (2019).
21. Gong, Y. et al. Ten years' experience with needle biopsy in the early diagnosis of sacroiliitis. *Arthritis Rheum.* **64**, 1399–1406 (2012).
22. Bakker, P. A. et al. Is it useful to repeat MRI of the sacroiliac joints after three months or one year in the diagnostic process of patients with chronic back pain suspected of axial spondyloarthritis? *Arthritis Rheumatol.* **71**, 382–391 (2019).
23. Sengupta, R. et al. Short-term repeat magnetic resonance imaging scans in suspected early axial spondyloarthritis are clinically relevant only in HLA-B27-positive male subjects. *J. Rheumatol.* **45**, 202–205 (2018).
24. Rusman, T. et al. Presence of active MRI lesions in patients suspected of non-radiographic axial spondyloarthritis with high disease activity and chance at conversion after a 6-month follow-up period. *Clin. Rheumatol.* **39**, 1521–1529 (2020).
25. van Onna, M. et al. HLA-B27 and gender independently determine the likelihood of a positive MRI of the sacroiliac joints in patients with early inflammatory back pain: a 2-year MRI follow-up study. *Ann. Rheum. Dis.* **70**, 1981–1985 (2011).
26. Landewe, R. et al. A single determination of C-reactive protein does not suffice to declare a patient with a diagnosis of axial spondyloarthritis 'CRP-negative'. *Arthritis Res. Ther.* **20**, 209 (2018).
27. Visser, M. et al. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* **282**, 2131–2135 (1999).
28. Aggarwal, R. et al. Distinctions between diagnostic and classification criteria? *Arthritis Care Res.* **67**, 891–897 (2015).
29. Khan, M. A. & van der Linden, S. Axial spondyloarthritis: a better name for an old disease: a step toward uniform reporting. *ACR Open Rheumatol.* **1**, 336–339 (2019).
30. Rudwaleit, M., Khan, M. A. & Sieper, J. The challenge of diagnosis and classification in early ankylosing spondylitis: do we need new criteria? *Arthritis Rheum.* **52**, 1000–1008 (2005).
31. Maksymowych, W. P. The role of imaging in the diagnosis and management of axial spondyloarthritis. *Nat. Rev. Rheumatol.* **15**, 657–672 (2019).
32. Feldtkeller, E. et al. Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. *Rheumatol. Int.* **23**, 61–66 (2003).
33. Seven, S. et al. Magnetic resonance imaging of lesions in the sacroiliac joints for differentiation of patients with axial spondyloarthritis from control subjects with or without pelvic or buttock pain: a prospective, cross-sectional study of 204 participants. *Arthritis Rheumatol.* **71**, 2034–2046 (2019).
34. de Winter, J. et al. Magnetic resonance imaging of the sacroiliac joints indicating sacroiliitis according to the Assessment of Spondyloarthritis International Society definition in healthy individuals, runners, and women with postpartum back pain. *Arthritis Rheumatol.* **70**, 1042–1048 (2018).
35. Baraliakos, X. et al. Frequency of MRI changes suggestive of axial spondyloarthritis in the axial

skeleton in a large population-based cohort of individuals aged <45 years. *Ann. Rheum. Dis.* **79**, 186–192 (2020).
36. Varkas, G. et al. Effect of mechanical stress on magnetic resonance imaging of the sacroiliac joints: assessment of military recruits by magnetic resonance imaging study. *Rheumatology* **57**, 508–513 (2018).
37. Weber, U. et al. Frequency and anatomic distribution of magnetic resonance imaging features in the sacroiliac joints of young athletes: exploring "background noise" toward a data-driven definition of sacroiliitis in early spondyloarthritis. *Arthritis Rheumatol.* **70**, 736–745 (2018).
38. Renson, T. et al. High prevalence of spondyloarthritis-like MRI lesions in postpartum women: a prospective analysis in relation to maternal, child and birth characteristics. *Ann. Rheum. Dis.* **79**, 929–934 (2020).
39. Bennett, P. & Wood, P. Population studies of the rheumatic diseases; Proceedings of the Third International Symposium, New York, June 5th–10th, 1966. (Excerpta Medica Foundation, 1968)
40. Kellgren, J., Jeffrey, M. & Ball, J. The epidemiology of chronic rheumatism. Vol. 1 (Blackwell Scientific Publications, 1963).
41. Khan, M. A. et al. Spondylitic disease without radiologic evidence of sacroiliitis in relatives of HLA-B27 positive ankylosing spondylitis patients. *Arthritis Rheum.* **28**, 40–43 (1985).
42. Sampaio-Barros, P. D. et al. Undifferentiated spondyloarthritis: a longterm followup. *J. Rheumatol.* **37**, 1195–1199 (2010).
43. Poddubnyy, D. et al. Rates and predictors of radiographic sacroiliitis progression over 2 years in patients with axial spondyloarthritis. *Ann. Rheum. Dis.* **70**, 1369–74 (2011).
44. Sany, J. et al. Unclassified HLA-B27 inflammatory rheumatic diseases: followup of 23 patients. *Arthritis Rheum.* **23**, 258–259 (1980).
45. Schattenkirchner, M. & Kruger, K. Natural course and prognosis of HLA-B27-positive oligoarthritis. *Clin. Rheumatol.* **6**, 83–86 (1987).
46. Mau, W. et al. Clinical features and prognosis of patients with possible ankylosing spondylitis. Results of a 10-year followup. *J. Rheumatol.* **15**, 1109–1114 (1988).
47. Oostveen, J. et al. Early detection of sacroiliitis on magnetic resonance imaging and subsequent development of sacroiliitis on plain radiography. A prospective, longitudinal study. *J. Rheumatol.* **26**, 1953–1958 (1999).
48. Bennett, A. N. et al. Severity of baseline magnetic resonance imaging-evident sacroiliitis and HLA-B27 status in early inflammatory back pain predict radiographically evident ankylosing spondylitis at eight years. *Arthritis Rheum.* **58**, 3413–3418 (2008).
49. Dougados, M. et al. Rate and predisposing factors for sacroiliac joint radiographic progression after a two-year follow-up period in recent-onset spondyloarthritis. *Arthritis Rheumatol.* **68**, 1904–1913 (2016).
50. Dougados, M. et al. Sacroiliac radiographic progression in recent onset axial spondyloarthritis: the 5-year data of the DESIR cohort. *Ann. Rheum. Dis.* **76**, 1823–1828 (2017).
51. Wang, R., Gabriel, S. E. & Ward, M. M. Progression of nonradiographic axial spondyloarthritis to ankylosing spondylitis: a population-based cohort study. *Arthritis Rheumatol.* **68**, 1415–1421 (2016).
52. Rudwaleit, M. et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. *Ann. Rheum. Dis.* **68**, 770–776 (2009).
53. van der Linden, S. et al. The ASAS criteria for axial spondyloarthritis: strengths, weaknesses, and proposals for a way forward. *Curr. Rheumatol. Rep.* **17**, 62 (2015).
54. Thomas, G. P. et al. Genetic diagnostic profiling in axial spondyloarthritis: a real world study. *Clin. Exp. Rheumatol.* **35**, 229–233 (2017).
55. Taylor, W. et al. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum.* **54**, 2665–73 (2006).
56. Rindskopf, D. & Rindskopf, W. The value of latent class analysis in medical diagnosis. *Stat. Med.* **5**, 21–27 (1986).
57. Sepriano, A. et al. What is axial spondyloarthritis? A latent class and transition analysis in the SPACE and DESIR cohorts. *Ann. Rheum. Dis.* **79**, 324–331 (2020).
58. US National Library of Medicine. ClinicalTrials.gov. CLassification of axial spondyloarthritis inception cohort (CLASSIC). https://clinicaltrials.gov/ct2/show/NCT03993847 (2020).
59. Hayward, R. J. & Machado, P. M. Classification criteria in axial spondyloarthritis: what have we learned; where are we going? *Rheum. Dis. Clin. North Am.* **46**, 259–274 (2020).
60. Dimitroulas, T. et al. Biologic drugs as analgesics for the management of low back pain and sciatica. *Pain. Med.* **20**, 1678–1686 (2019).
61. Dougados, M. et al. Symptomatic efficacy of etanercept and its effects on objective signs of inflammation in early nonradiographic axial spondyloarthritis: a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheumatol.* **66**, 2091–2102 (2014).
62. Brown, M. A. et al. Evaluation of the effect of baseline MRI sacroiliitis and C reactive protein status on etanercept treatment response in non-radiographic axial spondyloarthritis: a post hoc analysis of the EMBARK study. *Ann. Rheum. Dis.* **77**, 1091–1093 (2018).
63. Sieper, J. et al. A randomized, double-blind, placebo-controlled, sixteen-week study of subcutaneous golimumab in patients with active nonradiographic axial spondyloarthritis. *Arthritis Rheumatol.* **67**, 2702–2712 (2015).
64. Sieper, J. et al. Efficacy and safety of adalimumab in patients with non-radiographic axial spondyloarthritis: results of a randomised placebo-controlled trial (ABILITY-1). *Ann. Rheum. Dis.* **72**, 815–822 (2013).
65. Pharmaceutical Benefits Advisory Committee. Public summary document — November 2017 PBAC meeting. http://www.pbs.gov.au/industry/listing/elements/pbac-meetings/psd/2017-11/files/golimumab-nraxSpA-psd-november-2017.pdf (2017).
66. National Institute for Health and Care Excellence. Golimumab for treating non-radiographic axial spondyloarthritis. https://www.nice.org.uk/guidance/TA497 (2018)
67. Deodhar, A. et al. The concept of axial spondyloarthritis: joint statement of the spondyloarthritis research and treatment network and the Assessment of SpondyloArthritis international Society in response to the US Food and Drug Administration's comments and concerns. *Arthritis Rheumatol.* **66**, 2649–2656 (2014).
68. Landewe, R. et al. Efficacy of certolizumab pegol on signs and symptoms of axial spondyloarthritis including ankylosing spondylitis: 24-week results of a double-blind randomised placebo-controlled phase 3 study. *Ann. Rheum. Dis.* **73**, 39–47 (2014).
69. Sieper, J. Different approaches to drug approval by EMA and FDA — the example of non-radiographic axial spondyloarthritis [SP0108]. *Ann. Rheum. Dis.* **75**, 27 (2016).
70. Deodhar, A. et al. A fifty-two-week, randomized, placebo-controlled trial of certolizumab pegol in nonradiographic axial spondyloarthritis. *Arthritis Rheumatol.* **71**, 1101–1111 (2019).
71. Robinson, P. C. et al. Axial spondyloarthritis: a new disease entity, not necessarily early ankylosing spondylitis. *Ann. Rheum. Dis.* **72**, 162–164 (2013).
72. Akkoc, N. & Khan, M. A. Looking into the new ASAS classification criteria for axial spondyloarthritis through the other side of the glass. *Curr. Rheumatol. Rep.* **17**, 515 (2015).
73. Braun, J. et al. Classification and diagnosis of axial spondyloarthritis — what is the clinically relevant difference? *J. Rheumatol.* **42**, 31–38 (2015).
74. Robinson, P. C., Sengupta, R. & Siebert, S. Non-radiographic axial spondyloarthritis (nr-axSpA): advances in classification, imaging and therapy. *Rheumatol. Ther.* **6**, 165–177 (2019).
75. Proft, F. & Poddubnyy, D. Ankylosing spondylitis and axial spondyloarthritis: recent insights and impact of new classification criteria. *Ther. Adv. Musculoskelet. Dis.* **10**, 129–139 (2018).
76. Renson, T. et al. High prevalence of sacroiliac bone marrow edema on MRI in post partum women: a temporary phenomenom [abstract]. *Arthritis Rheumatol.* **71**, 597 (2019).
77. Lambert, R. G. et al. Defining active sacroiliitis on MRI for classification of axial spondyloarthritis: update by the ASAS MRI working group. *Ann. Rheum. Dis.* **75**, 1958–1963 (2016).
78. Lukas, C. et al. MRI for diagnosis of axial spondyloarthritis: major advance with critical limitations 'Not everything that glisters is gold (standard)'. *RMD Open* **4**, e000586 (2018).
79. van Tubergen, A. & Weber, U. Diagnosis and classification in spondyloarthritis: identifying a chameleon. *Nat. Rev. Rheumatol.* **8**, 253–261 (2012).
80. Bradbury, L. A. et al. Diffusion-weighted imaging is a sensitive and specific magnetic resonance sequence in the diagnosis of ankylosing spondylitis. *J. Rheumatol.* **45**, 771–778 (2018).
81. Weber, U. et al. Development and validation of a magnetic resonance imaging reference criterion for defining a positive sacroiliac joint magnetic resonance imaging finding in spondyloarthritis. *Arthritis Care Res.* **65**, 977–85 (2013).
82. Weber, U. et al. Assessment of structural lesions in sacroiliac joints enhances diagnostic utility of magnetic resonance imaging in early spondylarthritis. *Arthritis Care Res.* **62**, 1763–71 (2010).
83. Weber, U. et al. MRI of the sacroiliac joints in athletes: recognition of non-specific bone marrow oedema by semi-axial added to standard semi-coronal scans. *Rheumatology* **59**, 1381–1390 (2020).
84. van der Heijde, D. et al. Spinal inflammation in the absence of sacroiliac joint inflammation on magnetic resonance imaging in patients with active nonradiographic axial spondyloarthritis. *Arthritis Rheumatol.* **66**, 667–73 (2014).
85. Weber, U. et al. Does spinal MRI add incremental diagnostic value to MRI of the sacroiliac joints alone in patients with non-radiographic axial spondyloarthritis? *Ann. Rheum. Dis.* **74**, 985–92 (2015).
86. Weber, U. et al. Diagnostic utility of candidate definitions for demonstrating axial spondyloarthritis on magnetic resonance imaging of the spine. *Arthritis Rheumatol.* **67**, 924–33 (2015).
87. Costello, M. E. et al. Brief report: intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol.* **67**, 686–691 (2015).
88. Costello, M. E. et al. The intestinal microbiome in human disease and how it relates to arthritis and spondyloarthritis. *Best Pract. Res. Clin. Rheumatol.* **29**, 202–212 (2015).
89. Arnbak, B. et al. Associations between spondyloarthritis features and magnetic resonance imaging findings: a cross-sectional analysis of 1,020 patients with persistent low back pain. *Arthritis Rheumatol.* **68**, 892–900 (2016).
90. Markus, K. A. Constructs, concepts and the worlds of possibility: connecting the measurement, manipulation, and meaning of variables. *Measurement* **6**, 54–77 (2008).
91. Taylor, W. & Fransen, J. Distinctions between diagnostic and classification criteria: comment on the article by Aggawal et al. *Arthritis Care Res.* **68**, 149–150 (2015).
92. Dragulinescu, S. Diseases as natural kinds. *Theor. Med. Bioeth.* **31**, 347–69 (2010).
93. Williams, N. E. Arthritis and nature's joints, in Carving Nature at its joints. in Natural Kinds in Metaphysics and Science (eds Campbell, J. K, O'Rourke, M. & Slater, M. H.) (MIT Press, 2011).
94. Robinson, P. C. et al. Genetic dissection of acute anterior uveitis reveals similarities and differences in associations observed with ankylosing spondylitis. *Arthritis Rheumatol.* **67**, 140–151 (2015).

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Key opinion leaders — a critical perspective

Jose U. Scher and Georg Schett

Abstract | Enormous progress has been made in the field of rheumatology in the past several decades, historically led by publicly funded academic innovators but in more recent times with much greater involvement of the pharmaceutical industry. This shift in resources has created a complex new model for reinvestment in the medical community in which the vast majority of private funds are redirected towards influencing the prescription behaviour of practitioners through 'key opinion leaders', with the main purpose of enhancing and perpetuating profit rather than innovation and critical thinking, and often at the expense of partnerships with scientists (that is, basic and translational researchers) and academic collaborations. This new episteme brings multiple opportunities to rethink approaches to sustaining long-term critical research in the field, ultimately maximizing the return on investment: scientific knowledge for the benefit of patients and society. Central to such strategies should be the rebalancing of academia–industry partnerships towards academic research and the involvement of 'innovation and knowledge leaders', rather than mostly key opinion leaders.

During the course of the past century, biomedical research has brought about previously unimaginable advances in the understanding of disease pathogenesis and therapeutics and, with these advances, a dramatic reduction in deadly infections and uncontrollable inflammatory processes. The field of rheumatology has been at the forefront of this revolution, owing in large part to the ingenuity of astute clinicians and brilliant scientists backed mostly by federal funding. Within the past three decades, another paradigm shift in the treatment of chronic immune-mediated inflammatory diseases was led by the advent of monoclonal antibody technology and other modulators of specific immune pathways. These discoveries, supported by academic innovation, were soon expanded (rather exponentially) by the pharmaceutical industry, which generated a rapid accumulation of unparalleled financial wealth and resources by private companies at a time when public funding has been stalling[1].

Progress in rheumatology, as in all other fields of medicine, is dependent on the vital interaction between academic science and industry engaged in the arena. The dynamics of this relationship are complex and not always guided by the motivation to enhance knowledge and the development of improved therapeutics. The interaction between academia and industry also includes sophisticated methods that allow for the efficient spreading of opinions that can ultimately alter the prescribing patterns of physicians. In this article, we address the phenomenon of the key opinion leader (KOL), a steadily growing (in both number and influence) entity at the interface between academia and industry. Although serving as the primary nexus between companies and physicians and as a source of potentially valuable clinical information, the overall primary focus of KOLs is arguably aligned with the amplification goal of commercially driven interests. We discuss the challenges and conflicts that have emerged as a consequence of the current paradigm governing academia–industry interactions and question the pre-eminence of opinion-based leadership (that is, the KOL) at the expense of leadership based on innovation and knowledge. Finally, we present concepts and strategies to foster collaborations and expand knowledge that could lead to the discovery of novel targets for diagnostics and therapeutics in rheumatology, for the benefit of individual patients and society at large.

The birth of the KOL

In 1942, at the time when Nanna Svartz was publishing her results on the first rationally designed drug for the treatment of rheumatoid arthritis[2], the communications theorist Paul Lazarsfeld was busy trying to take the science of marketing to a whole new level. Lazarsfeld was sceptical about how much the mass (that is, direct-to-consumer) media truly shaped the public's views. In the course of his research into how Ohio voters actually changed their minds during the 1940 presidential election[3], Lazarsfeld discovered that human beings altered their views and preferences more because of trusted figures in their networks — or 'opinion leaders' — than because of forces such as advertising. His later work with Elihu Katz[4] elaborated on their 'two-step flow of communication' theory, which suggests that opinion leaders pay close attention to the mass media and pass on their interpretation of media messages to others.

By the mid-1950s, Lazarsfeld's group had extended their argument into medicine, through a study contracted by Pfizer about the factors that influenced doctors in the USA to adopt a new drug. In this landmark study[5], the authors asked the fundamental question that continues to drive every pharmaceutical marketing operation to this day: "What were the social processes that intervened between the initial trials of the drug by a few local innovators and its final use by virtually the whole medical community?" The simple answer: the implementation of a new drug is all about promoting and expanding "the effectiveness of interpersonal relations at each stage of the diffusion process".

Thus, the concept of the KOL in medicine was born. Since then, pharmaceutical companies have continually expanded their use of the KOL model of communication. According to the Pharma Marketing Network, KOLs are physicians or non-physician scientists who are engaged by pharmaceutical companies

to act as consultants to the companies but also to influence doctors' medical practice, including (but not limited to) their prescribing behaviour[6]. A catchier description of a KOL is suggested in an article in *The Chronicle of Higher Education* by Carl Elliott, who stated that "The KOL is a combination of celebrity spokesperson, neighbourhood gossip and the popular kid in high school"[7]. This latter description can be attributed to the fact that industry and KOLs often develop a kind of symbiotic relationship: industry feeds the hunger of the KOL for status and ego boosting, which motivates academic scientists to work for industry, and simultaneously KOLs support industry in the marketing of their products. It is therefore not surprising that the most desirable quality for a KOL is not always a scientist's knowledge or original innovative work, but rather other factors such as prescribing habits, memberships in organizations and contributions to treatment recommendations[8]. As outlined in TABLE 1, the fundamental function of a KOL is to act as an influencer rather than as a critical thinker; hence, the KOL acts primarily and necessarily as a marketing entity. Kimberly Elliott, an experienced former drug company sales representative, argued that "[KOLs] were sales people for us, and we would routinely measure the return on our investment, by tracking prescriptions before and after their presentations… If that speaker did not make the impact the company was looking for, then you would not invite them back"[9]. Thus, meetings, advisory boards and other events are important tools for setting up, expanding and publicizing the results of the symbiotic industry–KOL relationship. In 2008, the rheumatologist Ted Pincus coined the term 'hotel-based medicine', suggesting that some of the myriad scientific meetings might not primarily serve the well-intentioned purpose of expanding critical knowledge but rather represent marketing vehicles for specific products[10].

The two faces of the KOL

Towards the end of his life, the painter Diego Velazquez created his masterpiece *Las Meninas*, one of the most celebrated and yet complex paintings of modern times[11]. This enigmatic composition raises questions about reality and illusion, and the active and the passive, ultimately creating a perplexing relationship between the viewer and the figures depicted. In *Las Meninas*, we are unsure who is the viewer: are we watching Velazquez working or is he using us as a model? The intricate arrangement of sightlines, hiddenness and appearance in this piece of art confuses the viewer and might remind us in some ways of the KOL entity, who usually conveys commercially relevant content in an academic shroud. Conceptually, this camouflage (as in the case of the royal family depicted in *Las Meninas*) elevates the value and credibility of the content and represents a subliminal and efficacious strategy for reaching physician-customers, and is often complemented by other marketing strategies, including most notably dinners in high-end venues and the over-embellished industry booths found at major rheumatology meetings.

These and related approaches are commonly used in lectures (prepared by industry but presented by the KOL) and clinical studies (executed by companies but 'authored' by KOLs and ghostwriters)[8]. Concomitantly, however, the KOL has to successfully cultivate an aura of independence. Performing such a balancing act and successfully wrapping commercial content in scientific packaging is the ultimate talent of the KOL. In a different context, these skills have been described as the 'Dr Fox effect', coined from an experiment (c. 1970) in which a lecturer's expressiveness and their being labelled an 'expert', rather than the actual content of their lecture, affected students' learning behaviour[12]. Hence, influencers lacking even minimal personal contributions to the matter at hand can effectively disseminate their opinions as well as the interests of their circumstantial sponsors. Today, such opinion-based influence has gained further relevance owing to the amplification power of social media. Examples from the past few years include misrepresentation of the benefits of dietary products[13] as well as the unfounded hype for hydroxychloroquine as a treatment for COVID-19 (REF.[14]).

It is therefore not surprising to observe booming consulting enterprises (such as H1 or Global Vision Technology[15,16]) whose ultimate goal is to identify KOLs as well as to extract the critical information surrounding KOLs. For instance, these businesses claim to help "identify, analyse and apply the critical information surrounding thought leaders"[17] or to "help guide marketers to optimize KOL engagements as bona fide advisers to a brand and can help shape clinical development and clinical data publication plans"[16]. The companies use software incorporating artificial intelligence algorithms in order to identify and engage a 'personalized' roster of KOLs that provide advocacy and feedback for a pharmaceutical company, ultimately helping to create marketing strategies for that company's products.

Table 1 | **Comparison of IKLs and traditional KOLs**

Characteristic	IKL	KOL
Function	Scientist	Influencer
Aim	Gain of knowledge	Gain of influence
Motivation	Hunger for knowledge	Hunger for status
Key process	Innovation	Implementation
Data source	Own	External
Data handling	Data generation	Data dissemination
Concept	To be devised/conceived	Pre-formed
Instrument	Experiment	Steering committee
Study type	Investigator-initiated	Industry-sponsored
Project	Public initiative	Industry symposium
Involvement with industry	Early- and late-stage research	Late-stage research — post asset approval
Drug analogy	Originator	Biosimilar
Newspaper section analogy	Front page	Opinion page

IKL, innovation and knowledge leader; KOL, key opinion leader.

Invest in opinion or innovation?

The substantial interest in identifying KOLs illustrates the extent of investment by industry into the 'KOL community'. We seemingly live in times in which the dissemination of opinion is considered more desirable than investment in science-driven knowledge. Although this approach might be helpful in the short term (for example, by increasing sales and gaining market share), the mid-term and long-term consequences for rheumatology and other biomedical fields can be negative and ultimately inefficient, as industry ventures into the

mechanistic understanding of chronic diseases and the identification of new targets are at least partially cannibalized by commercial priorities[18]. The monetary value of pharmaceutical industry engagement of KOLs is best illustrated by data released under the US Open Payments programme of the Physician Payments Sunshine Act, which show that in 2018 companies made payments to ~627,000 physicians totalling over US$9.35 billion towards speaker and/or consulting fees or for the cumulative value of ownership interests[19]. These numbers are even more staggering when one considers that the entire NIH budget for 2018 was less than US$40 billion[20].

Notably, despite its budget stagnating over the past two decades, the NIH continues to have an important role in spurring private success[21,22], as highlighted by the modern endogenous growth theory, which underscores the importance of 'knowledge spillovers' for long-term economic growth. In his seminal paper[23], the 2018 Nobel Laureate in Economics Paul Romer argued that these knowledge spillovers (that is, when recipient entities and the economy as a whole gain material and intellectual capital that has been originally developed by others) mean that private firms (particularly pharmaceutical companies) underinvest in the production of knowledge. As a consequence, marketing-dominated strategies fuelled by an excess of opinions can lead to long-term negative consequences in overall health-care outcomes. Two types of policies are aimed at ameliorating this 'market failure': patent outcomes and public funding of research. A 2019 paper examined the effects of public science on private-sector innovation in the life sciences, and came up with relevant quantitative data[21]. For example, for each $10 million invested by the NIH in a research area, there are 2.7 associated private-sector patents in that field. Similarly, it has been established that $1 in NIH funding generates around $2.34 in drug sales[22]. Therefore, and because public-sector research is crucial for private-sector innovation, it is to be expected that at least a sizeable proportion of the revenue from industry would be invested back in basic fundamental knowledge of the disease mechanism. Hence, rather than investing in opinion-multiplication by KOLs, it seems strategically wise, and sustainable in the long run, for industry to rebalance its funding towards academic research.

At present, industry contributes to 5.9% of academic research in the USA[24]. Research programmes aimed at addressing a specific challenge and that can have immediate applicability (that is, falling within Pasteur's quadrant or use-inspired basic research) seem to receive funding from industry more often than either purely basic or applied research[25]. The proportion of university research funding provided by industry can vary substantially, ranging from as low as 1% and up to 22% for a single institution[24]. Although the concern that industry funding might jeopardize the productivity of scientists is a valid one[26], the most important channel for knowledge transfer from science to industry is in fact through the publication of research results[27]. Furthermore, private–public partnerships have not been shown to negatively affect academic freedom[28]. Critically, such initiatives enhance important indicators of innovation such as the generation of intellectual property, technology output, and numbers of jobs in high-tech sectors and new business start-ups, as well as venture capital acquisition[29,30].

Rethinking the current paradigm

Importantly, even when well-intended, the two-step flow of communication model has many inherent conflicts that necessarily lead to often blurry and difficult-to-regulate relationships between KOLs, their industry benefactors and the ultimate recipients of the primary message. The Sunshine Act and the mechanisms for disclosure and constraint of competing interests put forward by academic institutions have lessened the potential for larger conflicts. However, these stricter rules of engagement do not apply to the majority of prescribers to whom the payments are directed, or to recipients of major industry funding in university centres who 'forget' to disclose their financial conflicts[31–33].

The paradox, of course, is that the authors of this Perspective are subject to the same ethical dilemmas and potential conflicts when, as academic translational scientists, we willingly interact with industry partners. The phenotypic spectrum of reactions to these challenges ranges from a puristic strategy of complete and unconditional non-engagement to a wholly laissez-faire approach without much consideration for the implications (a behaviour that inevitably generates biases and conflicts, both conscious and unconscious). One could certainly argue that the former approach is the preferable one as it has several advantages when it comes to independence and transparency. However, given the realities we have described, we believe in an intermediate, more holistic approach that has clear, well-defined rules and that is conditional on the pursuit of goals higher than personal gain.

We therefore advocate for (and practice) increased transparency in statements of competing interests (both financial and related to intellectual property) and a more rigorous clarification of funders' and sponsors' roles within the dissemination process, including presentations at scientific meetings and the publication of peer-reviewed primary research data, treatment guidelines and review articles. We also think that academicians should participate mostly — if not exclusively — in upstream scientific discussions and collaborations in which both the content and the outcomes are not responsive to (or controlled by) the sponsors (that is, advisory boards should be reserved for honest discussions about potentially available therapeutic assets and study designs but not as a vehicle to 'shape the message' of a given product). Importantly, when contributing to educational activities, the content and its presentation should be fully developed by the investigators in an independent manner and without the participation of any industry representative, whether medical or commercial.

To be clear, we are not proposing that the ultimate authority of knowledge belongs to a select group of researchers and truth can only be attained exclusively through the application of the scientific method to unsolved problems. Our overall point is not to necessarily give pre-eminence to hard science over qualitative or multicultural research, but rather to restore its value in general and particularly in the specific interactions between physicians, researchers and health-care providers. We are saying that the current paradigm will necessarily be prone to self-perpetuating bias, misinformation and a consequent lack of progress should it continue its march towards an asymmetric dialogue in which the discourse is heavily dominated by less rigorous, non-evidence-based, opinion-driven dissemination of medical content.

Consequently, and based on the outlined challenges and observations, we believe that in order to advance the field, innovative models that integrate a wide range of applied basic, clinical and translational knowledge are needed in order to synergize the many inherent strengths (that is, human and intellectual capital) available across industry and academia.

Solutions and future prospects

The French philosopher Michel Foucault published *The Order of Things: An Archaeology of the Human Sciences*[34], in which he arrives at his central premise

that each historical period contains certain underlying epistemological assumptions that determine what is acceptable as scientific discourse. The episteme of the current scientific period remains to be elucidated, but it is certainly one that has so far brought an unprecedented understanding of the human body and its molecular and cellular networks through an ongoing liaison between bio-mathematical modelling and biomedicine that enables the understanding of complex structures and systems. Simultaneously, however, our era is marked by complexities and ambiguities. In today's paradigm, academic institutions mutate into corporate endeavours with return-on-investment as their mantra for progress and survival, whereas for-profit pharmaceutical companies absorb some of the brightest minds in the field.

Given these new realities, the time seems ripe to reconsider the partnership between investigators and industry. Furthermore, we are convinced that interactions between pharmaceutical companies and academia can be highly innovative, lead to new concepts in disease pathogenesis, and advance the fields of rheumatology and immunology. However, such interactions need to go far beyond opinion-based spreading of information, as is often mediated by the current KOL-driven model. A truly scientific dialogue between academic scientists and industry is needed more than ever, but these interactions could (and should) be re-balanced towards innovation-driven and data-driven science. We therefore propose that innovation and knowledge — rather than opinion — should constitute the foundation of the liaison between industry and academic scientists, and hence, an 'innovation and knowledge leader' (IKL), rather than a KOL, would be best suited for these interactions, ultimately leading to a long-term, mutually beneficial, innovation-driven, symbiotic relationship in pursuit of medical and scientific solutions for patients and society at large. TABLE 1 outlines the characteristics of the IKL and compares them with those of the traditional KOL.

Good examples are available in which interactions between IKLs and industry have led to outstanding advances in both immunology and rheumatology. In fact, breakthrough technological discoveries in immunology with relevance to rheumatology emerged from interdisciplinary collaborations of IKLs clustered in academia-like, science-driven institutions, which were founded (and funded) by private sources. Such innovations include hybridoma-based monoclonal antibody production at The Basel Institute of Immunology (funded by Hoffman La Roche)[35], the discovery and targeting of IL-23 at DNAX (funded by Schering Plough)[36] and the development of B cell-depleting strategies by targeting CD20 at Biogen IDEC[37]. Another notable example is the Immunology Catalyst Program designed by GlaxoSmithKline, which was dedicated to providing outstanding scientists with a 3-year sabbatical at the company's research and development hub with full access to compounds and technologies[38], or the joint venture approach illustrated by the Industry–University Cooperative Research Centers Program (IUCRC) in the USA. One can hope that similar initiatives will further proliferate, as they provide unique opportunities to study potentially interesting compounds 'on the shelf' that might be re-discovered, re-used and/or re-orientated in unexpected ways.

Furthermore, to enhance the understanding of the molecular pathogenesis of rheumatic diseases such as rheumatoid arthritis and systemic lupus erythematosus, multi-centre, multi-stakeholder, public–private partnerships have been established with the aim of better characterizing the molecular landscape of these diseases and of defining new treatment targets. For instance, the Accelerating Medicines Partnership (AMP) consortium in the USA is extraordinarily successful and has already served the joint interest of academia and industry to discover entirely new immune cell populations that orchestrate tissue inflammation, and has provided unbiased insights into human disease including the master regulators of the disease process[39,40]. Similarly, the Innovative Medicine Initiative (IMI) in Europe, which is funded by the European Union as well as partners from the European Federation of Pharmaceutical Industry (EFPIA), has made breakthroughs in understanding the molecular pathogenesis of rheumatic diseases and defining new treatment targets[41,42]. Without question, such partnerships would not be as fruitful if they lacked the high level of innovation that IKLs contribute to such projects. We are also convinced that, even when organizational skills are important for managing and executing such projects, these skills would not have the capacity to move the field forward and would most likely result in 'me too' projects if not paired with a critical level of innovation and knowledge (FIG. 1). Most importantly, and owing in large part to the 'honest broker' role of the NIH and European Union, such initiatives all but ensure that public funding and private investments from industry are mostly directed towards knowledge and innovation with little or no room for opinions.

Another productive way in which industry is currently supporting IKLs at academic institutions is by the awarding of grants through non-profit organizations such as the Rheumatology Research Foundation in the USA or the Foundation for Research in Rheumatology in Europe, as well as disease-specific non-profit organizations, such as the National Psoriasis Foundation, the Lupus Foundation of America and the Scleroderma Foundation, to name a few[43,44].

Thus, a number of instruments are already in place that strategically foster industry–academia cooperation for gaining scientific knowledge. Importantly, these instruments will require mechanisms to maintain and forward-feed these collaborative efforts. Recalibrating the partnership model towards the strengthening of engagement focused on knowledge and innovation is one such approach. However, these initiatives cannot (and should not) be driven exclusively by industry; endorsement by academic centres is equally critical. Take the case of academic recognition in relation to the development of clinical trials and dissemination of their results. Currently, investigators are almost entirely credited on the basis of authorship and citation metrics. However, such measurements do not distinguish whether the conceptualization of a given study, the source of the accrued data and/or the writing of a manuscript were the product of intellectual contributions by the scientist or if they were generated by the sponsor in totality. This reward system, which intermingles industry studies with academic recognition, ultimately supports an opinion-based KOL scheme that discourages the pursuit of more laborious, albeit independent and innovative, studies. However, such a system could be modified by academia in a way that values independent science and its own intellectual property while at the same time allowing industry to present their studies in a more authentic way. This modification could be achieved through various mechanisms, including the application of metric algorithms that place higher relative weight on independent contributions for academic promotions and departmental recognition.

Conclusions

In summary, we are cognizant of the ways in which economic forces are shaping the new scientific episteme and believe that

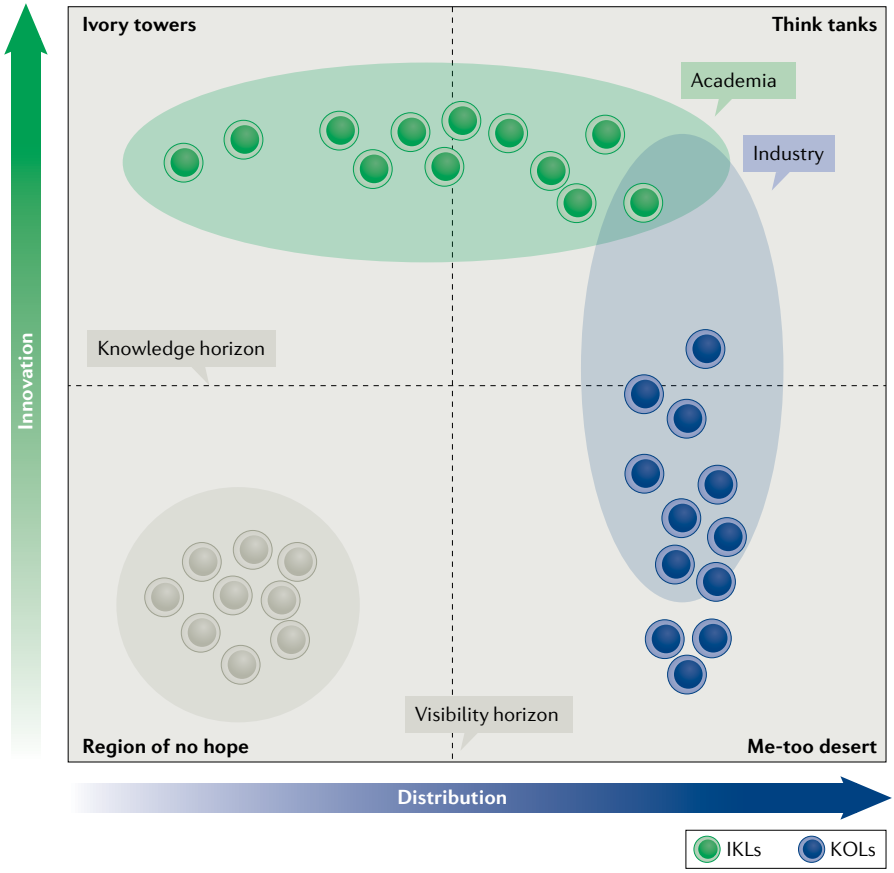

Fig. 1 | **Proposed Schett–Scher diagram for academia–industry collaboration.** The diagram shows the relationship between two main factors that dictate the interactions between academia and industry partners: innovation and distribution. Innovation is essential for gaining critical knowledge (knowledge horizon; dashed horizontal line), and distribution is essential for gaining visbility (visbility horizon; dashed vertical line). Academic institutions are traditionally (but not exclusively) knowledge oriented, whereas industry is mostly (but not exclusively) distribution driven. Models such as think tanks involving academia and industry require high-level and comprehensive knowledge and distribution (upper right panel). Key opinion leaders (KOLs) are always distribution-oriented but not necessarily knowledge-oriented, with some of them closely situated in the 'me-too desert' space (lower right panel). Innovation and knowledge leaders (IKLs) are spread across the upper two panels according to their different levels of distribution skills. In rare extremes, IKLs in 'ivory towers' have a great deal of knowledge but poor distribution skills (upper left panel). IKLs with better distribution skills, however, are well suited to sustained innovative academia–industry partnerships and collaboration.

the authors' voices can help to reorient the conversation towards critical thinking and innovation. We are firm defendants of the benefits that the enlightenment has brought to science over the past several decades and believe that academic–industry relations should move away from an opinion-driven, commercially immersed discussion and closer to a knowledge-generating, problem-solving cooperation. Unlike facts, opinions reflect personal statements based on values and beliefs and cannot definitively be proved or disproved by objective evidence. Although this opinionated discussion is acceptable and certainly admissible in liberal democracies founded around the concepts of liberty and freedom of speech, we should be careful that they do no dominate the discourse on any field, particularly in the sciences. Otherwise, we will surely lose all sense of factual, evidence-based, critical thinking that has provided so many advances for humankind. Efforts to reinvest in knowledge and innovation, rather than merely the dissemination of opinions, will enable the development of new ideas, which in turn will refresh the field and ultimately provide the basis for the mutually beneficial, long-term sustainability of immunology, rheumatology and the pharmaceutical industry alike.

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1. Burmester, G. R., Bijlsma, J. W. J., Cutolo, M. & McInnes, I. B. Managing rheumatic and musculoskeletal diseases — past, present and future. *Nat. Rev. Rheumatol.* **13**, 443–448 (2017).
2. Svartz, N. Salazopyrin, a new sulfanilamide preparation. A. Therapeutic results in rheumatic polyarthritis. B. Therapeutic results in ulcerative colitis. C. Toxic manifestations in treatment with sulfanilamide preparations. *Acta Med. Scand.* **110**, 577–598 (1942).
3. Lazarsfeld, P. F., Berelson, B. & Gaudet, H. *The People's Choice* (Duell, Sloan & Pearce, 1944).
4. Katz, E. & Lazarsfeld, P. F. *Personal Influence, The Part Played by People in the Flow of Mass Communications* (Transaction Publishers, 1966).
5. Coleman, J., Katz, E. & Menzel, H. The diffusion of an innovation among physicians. *Sociometry* **20**, 253–270 (1957).
6. Pharma Marketing Network. *The Pharma Marketing Glossary*. https://www.pharma-mkting.com/glossary/ (2020).
7. Elliott, C. *The Secret Lives of Big Pharma's 'Thought Leaders'* (The Chronicle of Higher Education, 2010).
8. Sah, S. & Fugh-Berman, A. Physicians under the influence: social psychology and industry marketing strategies. *J. Law Med. Ethics* **41**, 665–672 (2013).
9. Moynihan, R. Key opinion leaders: independent experts or drug representatives in disguise? *BMJ* **336**, 1402–1403 (2008).
10. Pincus, T., Bergman, M. J. & Yazici, Y. Hotel-based medicine. *J. Rheumatol.* **35**, 1487–1488 (2008).
11. Museo del Prado. Las Meninas https://www.museodelprado.es/en/the-collection/art-work/las-meninas/9fdc7800-9ade-48b0-ab8b-edee94ea877f (2019).
12. Naftulin, D. H., Ware, J. E. & Donnelly, F. A. The Doctor Fox lecture: a paradigm of educational seduction. *J. Med. Educ.* **48**, 630–635 (1973).
13. Tilburt, J. C., Allyse, M. & Hafferty, F. W. The case of Dr. Oz: ethics, evidence, and does professional self-regulation work? *AMA J. Ethics* **19**, 199–206 (2017).
14. Zagury-Orly, I. & Schwartzstein, R. M. Covid-19 — a reminder to reason. *N. Engl. J. Med.* **383**, e12 (2020).
15. H1. H1 Curie an Opinion Leader Identification and Analysis Platform by H1 https://www.h1insights.com/solutions/h1-curie (2020).
16. Global Vision Technology. CRM KOL Management https://www.global-visiontech.com/services-crm-services-crm-kol-management.html (2019).
17. GlobeNewswire. H1 To Incorporate Key Opinion Leader Data In Veeva CRM Platform https://www.globenewswire.com/news-release/2018/09/10/1568553/0/en/H1-To-Incorporate-Key-Opinion-Leader-Data-In-Veeva-CRM-Platform.html (2018).
18. Swanson, A. Big Pharmaceutical Companies are Spending Far More on Marketing than Research (The Washington Post, 2015).
19. Policy & Medicine. 2018 Open Payments Data Released https://www.policymed.com/2019/07/2018-open-payments-data-released.html (2019).
20. National Institutes of Health. Budget https://www.nih.gov/about-nih/what-we-do/budget (2020).
21. Azoulay, P., Graff Zivin, J. S., Li, D. & Sampat, B. N. Public R&D investments and private-sector patenting: evidence from NIH funding rules. *Rev. Econ. Stud.* **86**, 117–152 (2019).
22. NIH. The National Institutes of Health (NIH): Background and Congressional Issues. https://fas.org/sgp/crs/misc/R41705.pdf (2019).
23. Romer, P. M. The origins of endogenous growth. *J. Econ. Perspect.* **8**, 3–22 (1994).
24. Atkinson, R. D. Information Technology & Innovation Foundation. 2018. http://www2.itif.org/2018-industry-funding-university-research.pdf (2018).
25. Stokes, D. E. *Pasteur's Quadrant: Basic Science and Technological Innovation* (Brookings Institution Press, 2011).
26. Hottenrott, H. & Thorwarth, S. Industry funding of university research and scientific productivity. *Kyklos* **64**, 534–555 (2011).

27. Cohen, W. M., Nelson, R. R. & Walsh, J. P. Links and impacts: the influence of public research on industrial R&D. *Manag. Sci.* **48**, 1–23 (2002).
28. Gray, D. O. & Walters, S. G. *Managing the Industry/University Cooperative Research Center. A Guide for Directors and Other Stakeholders* (Battelle, 1998).
29. Atkinson, R. D. & Wu, J. J. *The 2017 State New Economy Index: Benchmarking Economic Transformation in the States* (SSRN, 2017).
30. National Science Foundation. National Science Foundation: National Science Board, Science & Engineering Indicators 2016 https://www.nsf.gov/statistics/2016/nsb20161/uploads/1/13/tt08-48.pdf (2016).
31. Rosenbaum, L. Beyond moral outrage — weighing the trade-offs of COI regulation. *N. Engl. J. Med.* **372**, 2064–2068 (2015).
32. Khan, N. A., Nguyen, C. L., Khawar, T., Spencer, H. & Torralba, K. D. Association of author's financial conflict of interest with characteristics and outcome of rheumatoid arthritis randomized controlled trials. *Rheumatology* **58**, 776–785 (2019).
33. Heneghan, C. & McCartney, M. Declaring interests and restoring trust in medicine. *BMJ* **367**, l6236 (2019).
34. Foucault, M. *The Order of Things: an Archaeology of the Human Sciences. 1966* (Vintage, 1973).
35. Köhler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
36. Oppmann, B. et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* **13**, 715–725 (2000).
37. Anderson, D. L. et al. UM Patent: Therapeutic application of chimeric antibodies to human B lymphocyte restricted differentiation antigen for treatment of B cell lymphoma. https://experts.umich.edu/details/patent/EP-1005870-A3 (2004).
38. Tkach Tuzman, K. Doctors in the house https://www.biocentury.com/article/291228/gsk-is-internalizing-external-innovation-bringing-academics-in-house (BioCentury Innovations, 2017).
39. Rao, D. A. et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* **542**, 110–114 (2017).
40. Croft, A. P. et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* **570**, 246–251 (2019).
41. Wohlfahrt, T. et al. PU.1 controls fibroblast polarization and tissue fibrosis. *Nature* **566**, 344–349 (2019).
42. Culemann, S. et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature* **572**, 670–675 (2019).
43. Davidson, A. & Polsky, D. Sustaining the rheumatology research enterprise. *Arthritis Care Res.* **67**, 1187 (2015).
44. Siegel, M., Shankle, L., Hwang, S. & Ogdie, A. The 2017 National Psoriasis Foundation research symposium. *J. Psoriasis Psoriatic Arthritis* **3**, 15–17 (2018).

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Author contributions

Both authors researched data for the article and substantially contributed to discussion of content, writing and review/editing of the manuscript before submission.

Competing interests

J.U.S. declares that he has served as a consultant for Janssen, Novartis, Pfizer, Sanofi and UCB, and has received funding for investigator-initiated studies from Novartis, Sanofi and Janssen. G.S. has served as a consultant for Abbvie, BMS, Eli Lilly, Gilead, GSK Novartis, Janssen and Roche and has received funding for investigator-initiated studies from BMS, Eli Lilly, GSK, Novartis and UCB.

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