Nature Reviews Rheumatology | Published online 7 Dec 2017; doi:10.1038/nrrheum.2017.202

THERAPY

Rheumatic symptoms associated with immune checkpoint inhibition

Rheumatic immune-related adverse events occur in some patients with cancer being treated with immune checkpoint inhibitors (ICIs). A new study by Kostine *et al.* highlights the wide variety of rheumatic immune-related adverse events that occur in such patients and suggests that the occurrence of these adverse events might predict a favourable response to ICI therapy.

Immune checkpoints are negative regulators of the immune response that promote tolerance and prevent excessive self-reactivity. However, tumour cells can use these immune checkpoints to evade the immune system. Inhibition of these checkpoints has been used as a revolutionary new therapy for many cancers. The ICIs currently approved for clinical use target either the cytotoxic T lymphocyte protein 4 (CTLA4) pathway or the programmed cell death protein 1 (PD1) pathway. However, immune checkpoint inhibition is also associated with various immune-related adverse events.

Kostine *et al.* sought to evaluate the prevalence of rheumatic immune-related adverse events in patients with cancer receiving ICI therapy. From a cohort of 524 such patients, 35 (6.6%) were referred to rheumatology services with any type of rheumatic symptoms; some patients had inflammatory arthritis (3.8%) that mimicked either rheumatoid arthritis (RA), polymyalgia rheumatica (PMR) or psoriatic arthritis, whereas others had non-inflammatory musculoskeletal conditions (2.8%). "While our study confirms that RA and PMR-like patterns are the most frequent rheumatic immune-related adverse events, the description of other clinical entities such as PMR-like patterns without increased levels of C-reactive protein, as well as non-inflammatory immune-related adverse events, is new and noteworthy for practicing rheumatologists," states corresponding author Marie Kostine.

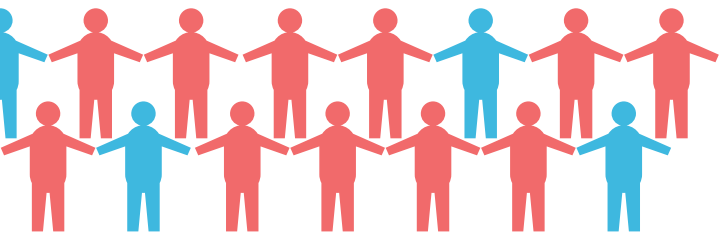
> "the occurrence of rheumatic immune-related adverse events could be a marker for an efficient and durable antitumour response"

For the majority of patients with inflammatory arthritis, low to moderate doses of glucocorticoids were sufficient to treat their musculoskeletal symptoms, with only two patients also requiring methotrexate therapy. By contrast, non-inflammatory musculoskeletal symptoms were effectively managed with NSAIDs, analgesics and/or physiotherapy. ICI treatment was continued in all but one patient, indicating that these adverse events can be effectively managed without the need to modify ICI treatment.

Of note, a higher proportion of patients with cancer who developed rheumatic immune-related adverse events were responsive to ICI treatment than those patients who did not develop rheumatic immune-related adverse events (85.7% vs 35.3%; $P < 0.0001$). These results are in line with the hypothesis that the occurrence of rheumatic immune-related adverse events could be a marker for an efficient and durable antitumour response. Furthermore, the majority of patients who presented with rheumatic immune-related adverse events were receiving anti-PD1 or anti-PD1 ligand 1 treatment, rather than anti-CTLA4 treatment, indicating that the onset of inflammatory arthritis might be related to the use of specific ICIs. Thierry Schaeverbeke, a co-author of the study, explains that the patients in this study will continue to be followed up to answer questions such as the long-term effect of these immunosuppressive strategies, to help to develop treatment algorithms and to investigate the phases of preclinical disease.

"This work emphasizes the need for a broad education among the rheumatologic community on rheumatic immune-related adverse events, the need for an international consensus on how to manage these complications, and especially the need for prospective studies to search for predictive biomarkers and to elucidate the basic underlying immunopathogenesis of these complications, of which our understanding is woefully incomplete," remarks Leonard Calabrese, who was not involved in the study.

Jessica McHugh



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ORIGINAL ARTICLE Kostine, M. *et al.* Rheumatic disorders associated with immune checkpoint inhibitors in patients with cancer — clinical aspects and relationship with tumour response: a single-centre prospective cohort study. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-212257 (2017)
FURTHER READING van der Vlist, M. *et al.* Immune checkpoints and rheumatic diseases: what can cancer immunotherapy teach us? *Nat. Rev. Rheumatol.* **12**, 593–604 (2016)

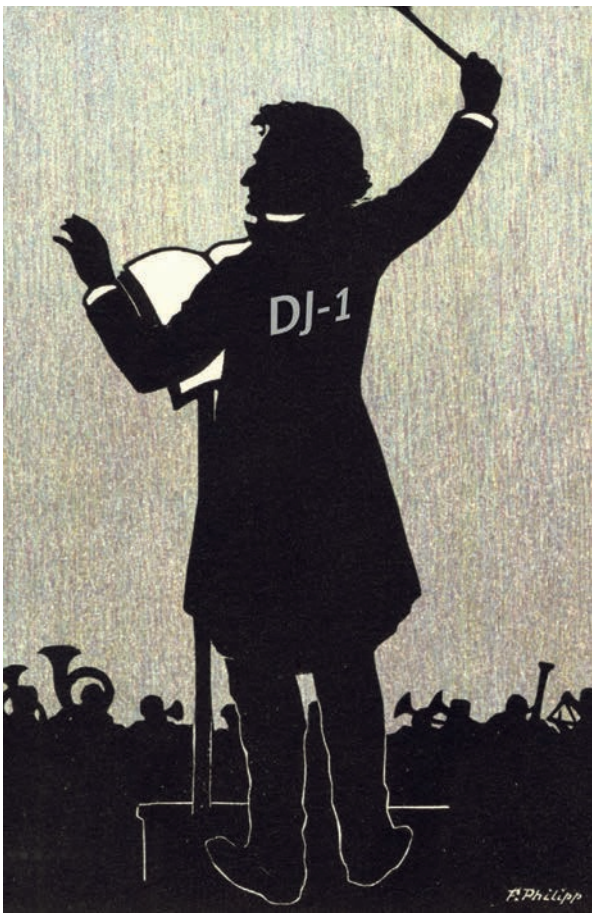
Nature Reviews Rheumatology | Published online 30 Nov 2017; doi:10.1038/nrrheum.2017.199

BONE

DJ-1 orchestrates osteoclastogenesis

> Interestingly, only male mice showed statistically significant levels of bone loss

The antioxidant protein/nucleic acid deglycase DJ-1 (encoded by *PARK7*), a reactive oxygen species (ROS) scavenger, has been linked to the development of cancer and early onset Parkinson disease.



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Despite ROS having a known role in osteoclastogenesis, the potential role of DJ-1 in this process had not been explored. Now, a new study has revealed the pivotal role of DJ-1 in regulating bone homeostasis.

"Our results indicate that DJ-1 is critical for normal physiological bone homeostasis," states corresponding author Wahn Soo Choi. "Its deficiency or dysfunction leads to overproduction of osteoclasts and eventually causes bone-associated diseases."

Choi and colleagues characterized the bone pathology of *Park7*$^{-/-}$ mice, which are deficient in DJ-1. Interestingly, only male mice showed statistically significant levels of bone loss by μCT imaging. These mice also had increased numbers of tartrate-resistant acid phosphatase type 5-positive osteoclasts (a marker of terminal osteoclast differentiation) compared with male wild-type mice.

In vitro, suppression of DJ-1 production using small interfering RNAs increased the differentiation of human CD14^{+} monocytes or murine bone marrow macrophages into osteoclasts. In comparison with bone marrow macrophages from wild-type mice, the same cells from *Park7*$^{-/-}$ mice produced an increased number of osteoclasts.

The researchers noticed high levels of signalling molecules and transcription factors usually associated with the receptor activator of nuclear factor-κB ligand (RANKL) signalling pathway in *Park7*$^{-/-}$ bone marrow macrophages. Treating these cells with a ROS scavenger reduced the levels of RANKL-activated signalling molecules to levels similar to those seen in wild-type bone marrow macrophages, suggesting that DJ-1 exerts its inhibitory effect on osteoclastogenesis by regulating levels of ROS.

Translating these findings into a disease setting, Choi and colleagues investigated the role of DJ-1 in collagen-induced arthritis (CIA), a model in which bone damage is caused by osteoclasts. Induction of CIA in *Park7*$^{-/-}$ mice produced higher levels of serum ROS than are found in wild-type mice with CIA. *Park7*$^{-/-}$ mice also had more severe disease, including increased synovial inflammation and bone erosion, than wild-type mice.

"On the basis of these results, we will conduct research to develop therapeutic methods to treat bone-associated diseases by controlling the function of DJ-1," concludes Choi.

Joanna Collison

ORIGINAL ARTICLE Kim, H. S. *et al.* DJ-1 controls bone homeostasis through the regulation of osteoclast differentiation. *Nat. Commun.* **8**, 1519 (2017)

Nature Reviews Rheumatology | Published online 16 Nov 2017; doi:10.1038/nrrheum2017.191

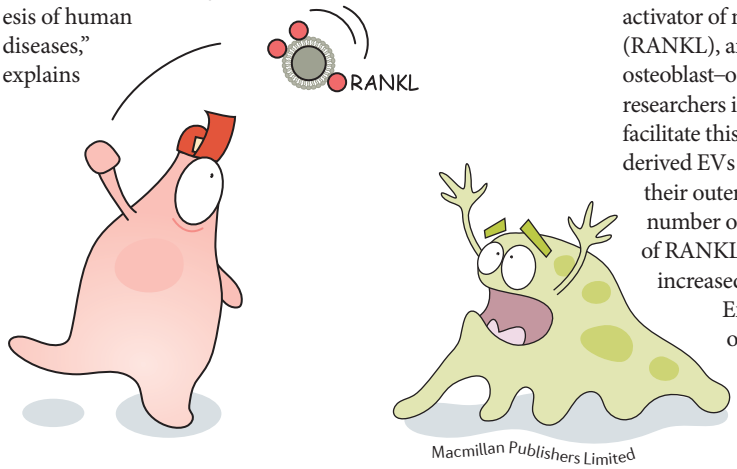
BONE

Extracellular vesicles in bone cell crosstalk

Extracellular vesicles (EVs) facilitate communication between many cell types and are currently being investigated for use as drug delivery systems; however, relatively little is known about their role in bone. A new study published in *The Journal of Bone and Mineral Research* has shed light on the role of EVs in osteoblast–osteoclast communication and also as a potential biotherapeutic technology.

"We started this project because we were attracted by the emerging field of EV research, which shows the potential to enable us to better understand the pathogenesis of human diseases," explains corresponding author Anna Teti. To explore the role of EVs in bone homeostasis, Teti and colleagues first established that EVs were produced by primary murine osteoblasts *in vitro*. These EVs conformed to standard morphology, could be easily harvested and were able to fuse with and transfer their contents to osteoblasts, monocytes and endothelial cells *in vitro*.

> "Osteoblast-derived [extracellular vesicles] contained RANKL in their outer membrane"



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Interestingly, the protein content of EVs increased when osteoblasts were pre-treated with parathyroid hormone (PTH), suggesting that the release of EVs is regulated hormonally. As PTH is a potent inducer of receptor activator of nuclear factor-κB ligand (RANKL), an important molecule in osteoblast–osteoclast crosstalk, the researchers investigated whether EVs facilitate this process. Osteoblast-derived EVs contained RANKL in their outer membrane, and both the number of vesicles and the amount of RANKL they contain were increased by treatment with PTH. Exposure of osteoclasts to osteoblast-derived EVs from wild-type mice increased osteoclast size and the number of nuclei per osteoclast — an effect not seen with osteoblast-derived EVs from *Rankl*$^{-/-}$ mice. *In vivo*, intraperitoneal injection of EVs from wild-type osteoblasts into *Rankl*$^{-/-}$ mice increased the presence of tartrate-resistant, acid phosphatase-positive cells in trabecular bone, which is indicative of neo-osteoclastogenesis.

In mice with retinoic acid-induced osteoclast overactivation, osteoblast-derived EVs pre-loaded with the anti-osteoclastic agents zoledronate or dasatinib reduced osteoclast activity to a similar degree to that seen with the free drugs, an effect that was absent when mice were treated with EVs alone. "We are currently extending our research to other bone diseases and hope to optimize the use of EVs as smart tools for the targeted delivery of regulatory factors and drugs," concludes Teti.

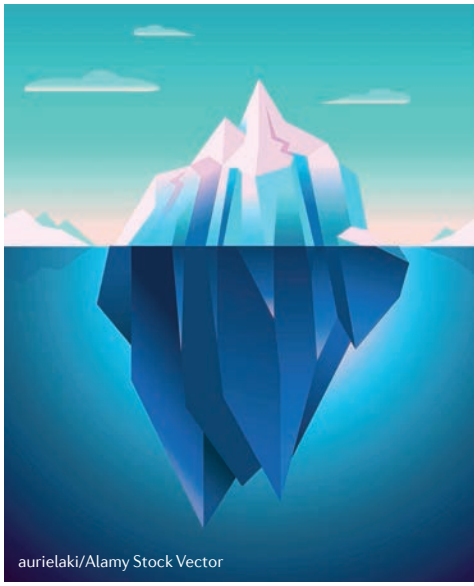
Joanna Collison

ORIGINAL ARTICLE Cappariello, A. *et al.* Osteoblast-derived extracellular vesicles are biological tools for the delivery of active molecules to bone. *J. Bone Miner. Res.* http://dx.doi.org/10.1002/jbmr.3332 (2017)
FURTHER READING Malda, J. *et al.* Extracellular vesicles — new tool for joint repair and regeneration. *Nat. Rev. Rheumatol.* **12**, 243–249 (2017)

Nature Reviews Rheumatology | Published online 19 Dec 2017; doi:10.1038/nrrheum.2017.203

EPIDEMIOLOGY

Arthritis more common than expected



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The prevalence of arthritis in the United States is much higher than previously estimated, particularly in younger adults, according to a new study published in *Arthritis & Rheumatology*. "Our findings mean that arthritis should not be perceived as a condition that only requires attention in the elderly population," states S. Reza Jafarzadeh, corresponding author of the study.

In the United States, estimates of the prevalence of arthritis commonly rely on self-report surveys such as the National Health Interview Survey (NHIS). The national estimate for the prevalence of arthritis in 2015 relied on a single question from the NHIS regarding whether an individual had physician-diagnosed arthritis and did not take into account other arthritis-related survey questions. To more accurately estimate the prevalence of arthritis, Jafarzadeh *et al.* developed surveillance criteria based on three NHIS questions relating to physician-diagnosed arthritis, chronic joint symptoms and the duration of these symptoms; to infer population parameters the researchers used statistical modelling. "Our analytic approach explicitly adjusts for the misclassification that is driven by the imperfect accuracy of the arthritis-related questions in the NHIS survey," explains Jafarzadeh. "This approach allows an estimation of the true prevalence of arthritis from aggregate-level data."

Using this approach, the prevalence of arthritis in 2015 was estimated at 36.8% in the US adult population (91.2 million adults of the 247.7 million projected total population), which was 68% higher than the previously reported 2015 national estimate of 22.7% (54.5 million adults). Furthermore, 30.6% of younger adults (individuals aged 18–64, 61.1 million adults) had arthritis. Of the three surveillance criteria, the physician-diagnosed arthritis criterion had the lowest sensitivity in the younger adult group when stratified for age and sex, whereas the duration of symptoms criterion had the highest sensitivity.

> "the prevalence of arthritis in 2015 was estimated at 36.8% in the US adult population"

"Our study suggests that arthritis prevalence in the US adult population has been substantially underestimated, especially in adults below 65 years of age," remarks Jafarzadeh. "Future research could focus on the better monitoring of arthritis and on increasing arthritis awareness and prevention, which should improve the wellbeing of the population, especially for younger adults."

Jessica McHugh

ORIGINAL ARTICLE Jafarzadeh, S. R. & Felson, D. T. Updated estimates suggest a much higher prevalence of arthritis in US adults than previous ones. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40355 (2017)

Nature Reviews Rheumatology | Published online 23 Nov 2017

IN BRIEF

CRYSTAL ARTHRITIS

Stepping up febuxostat to treat gout flares

A stepwise dose increase of febuxostat was comparable to prophylactic low-dose colchicine for reducing flares in an open-label, randomized study of 241 patients with gout. Flares occurred in 20.8% of patients taking stepped-up (10 mg to 40 mg daily) febuxostat and in 18.9% of patients taking 40 mg daily febuxostat with low-dose colchicine, incidences that were significantly lower than those seen in patients taking 40 mg daily febuxostat alone ($P = 0.047$ and $P = 0.024$, respectively).

ORIGINAL ARTICLE Yamanaka, H. *et al.* Stepwise dose increase of febuxostat is comparable with colchicine prophylaxis for the prevention of gout flares during the initial phase of urate-lowering therapy: results from FORTUNE-1, a prospective, multicentre randomised study. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211574 (2017)

OSTEOPOROSIS

Teriparatide preferable for fracture prevention

In a head-to-head trial of teriparatide versus risedronate in 1,360 post-menopausal women with severe osteoporosis (defined as having two moderate or one severe vertebral fractures and a bone mineral density T score of –1 or less), new vertebral fractures occurred in 5.4% of women taking teriparatide compared with 12% of those taking risedronate ($P < 0.0001$). Incidences of clinical fractures ($P = 0.0009$) were also reduced in the teriparatide group compared with the risedronate group.

ORIGINAL ARTICLE Kendler, D. L. *et al.* Effects of teriparatide and risedronate on new fractures in post-menopausal women with severe osteoporosis (VERO): a multicentre, double-blind, double-dummy, randomised controlled trial. *Lancet* http://dx.doi.org/10.1016/S0140-6736(17)32137-2 (2017)

SYSTEMIC LUPUS ERYTHEMATOSUS

Effects of disease activity on pregnancy outcomes

Comparison of data on births in women with systemic lupus erythematosus (SLE; $n = 180$) and in the general population in Norway ($n = 498{,}849$) has revealed links between disease activity and pregnancy outcomes. Patients with SLE had an increased risk of low birth weight in neonates ($P < 0.001$) and preterm birth ($P = 0.003$) compared with population controls, effects that were more pronounced in the setting of active disease. Patients with active disease also had an increased risk of pre-eclampsia compared with the general population or patients with inactive disease ($P < 0.001$ and $P = 0.052$, respectively).

ORIGINAL ARTICLE Götestam Skorpen, C. *et al.* Influence of disease activity and medications on offspring birth weight, pre-eclampsia and preterm birth in systemic lupus erythematosus: a population-based study. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211641 (2017)

RHEUMATOID ARTHRITIS

Tocilizumab prevents progression of bone erosions

Results from 317 newly diagnosed DMARD-naive patients with rheumatoid arthritis enrolled in the U-Act-Early trial show a clear reduction in the progression of bone erosions after 104 weeks of treatment with tocilizumab alone or in combination with methotrexate compared with treatment with methotrexate alone ($P \leq 0.023$). The proportion of patients who showed no progression of erosions was also higher among those taking tocilizumab than methotrexate alone at weeks 52 and 104.

ORIGINAL ARTICLE Teitsma, X. M. *et al.* Radiographic joint damage in early rheumatoid arthritis patients: comparing tocilizumab- and methotrexate-based treat-to-target strategies. *Rheumatology (Oxford)* http://dx.doi.org/10.1093/rheumatology/kex386 (2017)

Nature Reviews Rheumatology | Published online 19 Dec 2017; doi:10.1038/nrrheum.2017.183

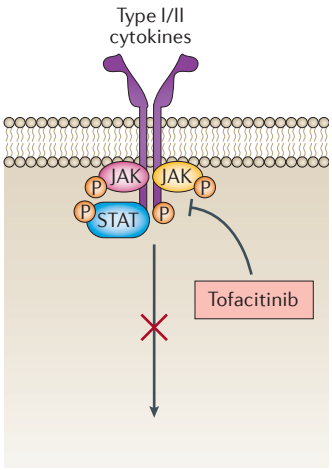
SPONDYLOARTHROPATHIES

Tofacitinib shows promise in PsA trials

> At 3 months, tofacitinib was superior to placebo with regard to both primary end points

The findings of two phase III trials, Oral Psoriatic Arthritis Trial (OPAL) Broaden and OPAL Beyond, support the use of the orally administered Janus kinase (JAK) inhibitor tofacitinib for the treatment of psoriatic arthritis (PsA).

"The reason for performing these studies was to test a new mechanism of action for the management of patients with PsA," explains Dafna Gladman, on behalf of the OPAL Beyond study authors. "Although TNF inhibitors as well as agents directed at IL-17 and IL-12/23 have been proved effective, these medications do not work for all patients, and may be toxic for some patients. Therefore, a drug with a different mechanism of action might work better," she continues.



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"The unmet need in PsA is high [now] and [was] especially several years ago when very few treatment options were available," adds Philip Mease, corresponding author of the OPAL Broaden trial. "Basic science data supported the concept that tofacitinib would demonstrate efficacy via inhibition (directly or indirectly) of multiple cytokines that are involved in PsA," he says.

OPAL Broaden enrolled 422 patients with PsA who had failed to respond to previous treatment with a TNF inhibitor, and OPAL Beyond involved 395 patients with PsA who had not previously been treated with a TNF inhibitor. In both studies, patients were randomly assigned to receive either tofacitinib 5 mg twice daily, tofacitinib 10 mg twice daily or placebo for 3 months; the OPAL Broaden trial also included a group who received an active control, adalimumab 40 mg every 2 weeks.

Notably, all patients enrolled in the studies were required to receive stable background treatment with a conventional synthetic DMARD, such as methotrexate, sulfasalazine or leflunomide. In addition, the placebo period was limited to 3 months, after which all patients in the placebo groups were switched to tofacitinib at either the 5 mg or the 10 mg twice daily dose. Each trial had a total duration of 6 months.

At 3 months, tofacitinib was superior to placebo with regard to both primary end points: the proportion of patients who achieved an ACR20 response (ACR criteria for ≥20% improvement) and change from baseline in the Health Assessment Questionnaire disability index (HAQ-DI) score.

"If approved, tofacitinib will expand the options available to patients with active PsA," says Mease. "It appears that the drug can be reasonably used [at] various points along the treatment path, including before biologics or after biologics have been tried," he adds. Further studies are needed to determine the long-term safety and efficacy of tofacitinib in PsA, and how it will be used alongside other available treatments.

Sarah Onuora

ORIGINAL ARTICLES Gladman, D. *et al.* Tofacitinib for psoriatic arthritis in patients with an inadequate response to TNF inhibitors. *N. Engl. J. Med.* **377**, 1525–1536 (2017) | Mease, P. *et al.* Tofacitinib or adalimumab versus placebo for psoriatic arthritis. *N. Engl. J. Med.* **377**, 1537–1550 (2017)

SPONDYLOARTHROPATHIES

TNF inhibitors and structural damage in ankylosing spondylitis

Atul Deodhar

Whether TNF inhibitors prevent structural damage in ankylosing spondylitis remains a controversial topic, as three prospective trials failed to show any evidence to support this notion. However, data are accumulating from retrospective analyses of well-characterized cohorts of patients that could provide the solution to this controversy.

Refers to Molnar C. *et al.* TNF blockers inhibit spinal radiographic progression in ankylosing spondylitis by reducing disease activity: results from the Swiss Clinical Quality Management cohort. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211544 (2017)

In rheumatology, the term 'disease modification' is used in a narrow sense to denote the prevention of structural damage as assessed by radiography, rather than to suggest change in the natural course of the disease that affects all manifestations of the condition, including mortality. Conventional synthetic DMARDs (for example, methotrexate, leflunomide and sulfasalazine) can be labelled as disease modifying owing to their ability to reduce the rate of progression of bone and cartilage damage compared with placebo, as observed by plain radiography in patients with rheumatoid arthritis (RA). None of these agents has been shown to prevent structural damage in psoriatic arthritis (PsA), although curiously they are still commonly referred to as DMARDs in PsA treatment. In ankylosing spondylitis (AS), a disease in which the predominant structural damage is caused by abnormal bone formation, these agents are not even termed 'axial symptom modifying', let alone disease modifying. The advent of TNF inhibitors at the turn of the last century has taken the concept of disease modification a step further. Not only is the progression of structural damage substantially reduced or even arrested in a large number of patients with RA who are taking TNF inhibitors, but cardiovascular mortality is also reduced in these patients, hinting at changes being made to the natural course of the disease. However, whether TNF inhibitors are disease modifying in patients with AS remains a controversial topic, as explored in a new study by Molnar *et al.*[1].

Open-label extensions of pivotal trials of etanercept[2], infliximab[3] and adalimumab[4] in AS showed success in modifying symptoms and in reducing disease activity but failed to show any effects on structural modification over 2 years. For ethical reasons, the placebo groups in each of these trials could not be continued beyond 6 months, so a group of patients with AS who had historically not used TNF inhibitors was used as controls for all three studies. In contrast to RA, in which bony erosions on hand radiographs can be seen as early as within 3 – 6 months of diagnosis, new bone formation in the axial skeleton occurs at a slow rate in patients with AS; therefore trials for AS need to be at least 2 years in duration. This long trial duration makes randomized placebo-controlled prospective trials in AS that specifically look for prevention of structural damage unethical and impossible to conduct. At least two possible solutions to this problem exist, the first being the use of more sensitive imaging techniques than plain radiography to assess changes in the axial skeleton, such as low-dose CT or MRI. Using these techniques, the conduction of a shorter and therefore ethically acceptable placebo-controlled prospective clinical trial might be possible. The second solution would be to retrospectively analyse longitudinally collected data from a well-characterized cohort of patients with AS; however, it should be remembered that only prospective randomized controlled trials can prove causality; retrospective analysis can only suggest associations.

> use of TNF inhibitors during the same 2-year radiographic interval did not prevent structural damage

Molnar *et al.*[1] attempted to answer the question of whether TNF inhibitors reduce spinal radiographic progression in AS by analysing 10-year follow-up data on patients from the Swiss Clinical Quality Management cohort. As in RA, inflammation is key to structural damage in AS; hence the effect of TNF inhibitors on radiographic progression is likely to be mediated through a reduction in disease activity, and to therefore take a long time to manifest. Molnar and colleagues[1] assessed radiographic progression at 2-year intervals and looked at the use of TNF inhibitors before these 2-year intervals. They found that prior use of TNF inhibitors reduced the odds of radiographic progression during the subsequent 2-year radiographic interval by 50% (OR 0.50, 95% CI 0.28–0.88). This effect seemed to be mediated by the inhibitory effect of TNF inhibitors on disease activity, as measured by the Ankylosing Spondylitis Disease Activity Score (ASDAS). Consistent with the results of previous studies, a high baseline damage score and male sex were associated with increased progression of structural damage, but contrary to previous experience, smoking, physical activity and use of NSAIDs had no effect on radiographic progression. Interestingly, the use of TNF inhibitors during the same 2-year radiographic interval did not prevent structural damage[1], which would explain the apparent failure of TNF inhibitors to slow structural damage in previous pivotal studies[2–4].

The results from Molnar *et al.*[1] add to accumulating evidence for an association between TNF inhibitor use and a reduction in spinal radiographic progression in AS, and are reassuringly similar to the results of three other retrospective studies on independent cohorts of patients from the USA and Canada[5], the Netherlands[6] and Germany[7]. The study from the USA and Canada found that TNF inhibitor treatment in patients with AS was associated with a 50% reduction in the odds of radiographic progression (OR 0.52, 95% CI 0.30–0.88)[5], whereas the Dutch and German studies both found that the progression of structural damage was linear for the first 4 years in patients with AS taking TNF inhibitors, and then progressed at a much lower rate between 4 years and 6 years[6], and between 4 years and 8 years[7]. Taken together, these data[1,5–7] are sufficient to prove an association between long-term use of TNF inhibitors and reduction in spinal radiographic progression in patients with AS; it is also clear that this effect is mediated by the ability of TNF inhibitors to control disease activity. Although a true cause and effect relationship can only be proved by prospective randomized controlled studies, this association has clear implications for daily clinical practice. The 2017 'treat-to-target' strategy for spondyloarthritis[8] and the Assessment of Spondyloarthritis International Society (ASAS)–EULAR treatment recommendations for axial spondyloarthritis[9] suggest that clinicians should aim to achieve inactive disease (ASDAS <1.3) in an attempt to reduce structural damage and improve long-term outcomes in patients with AS.

Within the past 2 years, the first in a new class of agents, the IL-17A inhibitor secukinumab, has been approved for the treatment of AS. A phase III study of secukinumab showed that spinal radiographic damage did not increase over 2 years in 80% of patients with AS, results that persisted after 4 years in an extension to the study[10]. Secukinumab also reduced disease activity in AS, but whether its effect on structural damage is mediated through suppression of disease activity, or through some other molecular mechanism, is unknown. A head-to-head study of secukinumab with a TNF inhibitor in patients with AS is currently being planned, the results of which might answer the question of which of these two modes of action (IL-17 inhibition or TNF inhibition) is more successful. The results of this study might also help clinicians to decide which biologic to choose when treating patients with AS who have an inadequate response to NSAIDs.

> a true cause and effect relationship can only be proved by prospective randomized controlled studies

Overall, to know whether biologics are truly disease modifying in AS, we need to take an expansive and holistic view. Data are needed on whether TNF inhibitors and IL-17 inhibitors positively affect extra-articular manifestations of AS such as uveitis, inflammatory bowel disease, peripheral arthritis, psoriasis and aortic root involvement, as well as comorbidities such as ischaemic heart disease and, ultimately, mortality. These answers can only be found through careful analyses of data collected in large cohorts.

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1. Molnar, C. *et al.* TNF blockers inhibit spinal radiographic progression in ankylosing spondylitis by reducing disease activity: results from the Swiss Clinical Quality Management cohort. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211544 (2017).
2. van der Heijde, D. *et al.* Radiographic progression of ankylosing spondylitis after up to two years of treatment with etanercept. *Arthritis Rheum.* **58**, 1324–1331 (2008).
3. van der Heijde, D. *et al.* Radiographic findings following two years of infliximab therapy in patients with ankylosing spondylitis. *Arthritis Rheum.* **58**, 3063–3070 (2008).
4. van der Heijde, D. *et al.* Assessment of radiographic progression in the spines of patients with ankylosing spondylitis treated with adalimumab for up to 2 years. *Arthritis Res. Ther.* **11**, R127 (2009).
5. Haroon, N. *et al.* The impact of tumor necrosis factor alpha inhibitors on radiographic progression in ankylosing spondylitis. *Arthritis Rheum.* **65**, 2645–2654 (2013).
6. Maas, F. *et al.* Reduction in spinal radiographic progression in ankylosing spondylitis patients receiving prolonged treatment with tumor necrosis factor inhibitors. *Arthritis Care Res.* **69**, 1011–1019 (2017).
7. Baraliakos, X. *et al.* Continuous long-term anti-TNF therapy does not lead to an increase in the rate of new bone formation over 8 years in patients with ankylosing spondylitis. *Ann. Rheum. Dis.* **73**, 710–715 (2014).
8. Smolen, J. S. *et al.* Treating axial spondyloarthritis and peripheral spondyloarthritis, especially psoriatic arthritis, to target: 2017 update of recommendations by an international task force. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211734 (2017).
9. van der Heijde, D. *et al.* 2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis. *Ann. Rheum. Dis.* **76**, 978–991 (2017).
10. Braun, J. *et al.* Secukinumab demonstrates low radiographic progression and sustained efficacy through 4 years in patients with active ankylosing spondylitis [abstract]. *Ann. Rheum. Dis.* **69** (suppl 10), 3L (2017).

Competing interests statement
A.D. declares that he has acted on the advisory board for or received research grants from Abbvie, Eli Lilly, Janssen, Novartis, Pfizer and UCB.

Autoimmunity and primary immunodeficiency: two sides of the same coin?

Reinhold E. Schmidt[1], Bodo Grimbacher[2] and Torsten Witte[1]

Abstract | Autoimmunity and immunodeficiency were previously considered to be mutually exclusive conditions; however, increased understanding of the complex immune regulatory and signalling mechanisms involved, coupled with the application of genetic analysis, is revealing the complex relationships between primary immunodeficiency syndromes and autoimmune diseases. Single-gene defects can cause rare diseases that predominantly present with autoimmune symptoms. Such genetic defects also predispose individuals to recurrent infections (a hallmark of immunodeficiency) and can cause primary immunodeficiencies, which can also lead to immune dysregulation and autoimmunity. Moreover, risk factors for polygenic rheumatic diseases often exist in the same genes as the mutations that give rise to primary immunodeficiency syndromes. In this Review, various primary immunodeficiency syndromes are presented, along with their pathogenetic mechanisms and relationship to autoimmune diseases, in an effort to increase awareness of immunodeficiencies that occur concurrently with autoimmune diseases and to highlight the need to initiate appropriate genetic tests. The growing knowledge of various genetically determined pathologic mechanisms in patients with immunodeficiencies who have autoimmune symptoms opens up new avenues for personalized molecular therapies that could potentially treat immunodeficiency and autoimmunity at the same time, and that could be further explored in the context of autoimmune rheumatic diseases.

The signs and symptoms of most rheumatic diseases are classified in international ACR or EULAR criteria; however, only weak hypotheses exist surrounding the pathogenesis of many of these inflammatory or autoimmune diseases. Over the past few years, an increase in research into the genetics behind these diseases has led to the detection of several genetic mutations that are linked to the immune dysregulation seen in many rheumatic diseases. A high degree of overlap exists between autoimmune diseases and primary immunodeficiencies in terms of genetic associations and causes; a 2017 analysis of the French National Primary Immunodeficiencies Registry (CEREDIH) showed that one or more autoimmune or inflammatory symptom was observed in 26.2% of patients with primary immunodeficiencies throughout their lifetime[1]. The risk of autoimmune cytopenia was also calculated to be at least 120 times higher in patients with primary immunodeficiencies than in the general population[1].

In this Review, we discuss the genetic and pathophysiological basis of a selection of primary immunodeficiency syndromes and describe the clinical autoimmune manifestations seen in these patients. These clinical features, along with knowledge of the relationship between autoimmunity and immunodeficiency, might be useful for differential diagnosis and lead to an increase in well-defined disease entities in the future. Moreover, the increasing use of selective biologic therapies and kinase inhibitors to treat rheumatic diseases can lead to a state of relative immunodeficiency in patients. Adverse effects of these therapies, including an increased risk of some infections, might be better understood once the relationships between these two groups of diseases are understood. This Review cannot cover all primary immunodeficiency syndromes, so we focus on those that are the most relevant to rheumatic diseases (TABLE 1).

Shared genetic risk factors

From a genetics point of view, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are currently the two best-studied autoimmune rheumatic diseases. Genome-wide association studies (GWAS) have identified 377 candidate genes in 100 non-MHC risk loci based on data from 29,880 patients with RA[2]. 98 of these genes

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Key points

- Immune dysregulation in many primary immunodeficiency syndromes leads to autoimmune disease manifestations
- Mutations in various genes can lead to immunodeficiencies, as well as to autoimmunity
- Specific knowledge of these genetic alterations and their pathophysiological consequences will enable the development of new therapeutic approaches
- Knowledge of primary immunodeficiency syndromes will enable a better understanding of potential infection-related adverse events when DMARDs are used to treat rheumatic diseases

were associated with a twofold increase in the risk of developing RA[2], 15 of which were identical to those previously associated with primary immunodeficiency syndromes[3]. These 15 genes encode molecules that are involved in multiple aspects of immune regulation, including caspase-8, caspase-10, autoimmune regulator (AIRE), IL-2 receptor α (also known as CD25), receptor-type tyrosine-protein phosphatase C (also known as CD45), VDJ recombination-activating protein 1 (RAG1), RAG2, CD40, serine-protein kinase ATM, non-receptor tyrosine-protein kinase TYK2 (TYK2), uracil-DNA glycosylase, IFNγ receptor 2 and interferon regulatory factor 8 (REF. 3).

In contrast to the polygenic traits associated with most rheumatic diseases, monogenic defects have been described in autoinflammatory diseases such as the periodic fever syndromes, which include familial Mediterranean fever, hyper-IgD with periodic fever syndrome, TNF receptor-associated periodic syndrome, deficiencies of IL-1 receptor antagonists and cryopyrin-associated periodic syndromes (also known as cryopyrinopathies). These periodic fever syndromes can all be accompanied by arthritis[3]. Given that some of these diseases have monogenic causes, it could be hypothesized that in the future more rheumatic diseases could be divided into clearly defined genetic diseases.

Similar to RA, SLE is associated with variants in several genes both within and outside of the MHC region. Among these variants are those in genes encoding the complement proteins C1q, C2 and C4, along with risk loci in another 50 non-MHC risk genes that have been identified by GWAS[4–6]. One group of genes includes variants that lead to gain-of-function (GOF) in the IFNα signalling pathway, which cause the so-called interferonopathies, and includes *TLR7*, *TLR8*, *TLR9*, *IRF3*, *IRF5*, *IRF7*, *TYK2*, *STAT4* and *IRAK1* (REF. 7). Variants of these genes contribute to an increase in IFNα production, a typical signature seen in patients with SLE, in whom the production of type I interferons (such as IFNα) is induced by RNA and single-stranded DNA[8]. Immune complexes containing these nucleic acid molecules can also stimulate the production of type I interferons, thereby promoting autoimmunity[9]. Increased levels of RNA and DNA can themselves be the result of genetic loss-of-function (LOF) mutations in *TREX1*, *RNASEH2A*, *SAMHD1*, *IFIH1* and *MAVS*, leading to subsequent type I interferon production and autoimmune disease[10–13].

Lymphocyte development and tolerance

The development of B cells and T cells requires the rearrangement and recombination of immunoglobulin and T cell receptor (TCR) genes, with the latter process taking place in the thymus. The recombinase genes *RAG1* and *RAG2* are critical for VDJ recombination and therefore for the development of lymphocytes: together with other enzymes and recombinases, RAG1 and RAG2 form a complex that initiates DNA cleavage and then repairs DNA breaks during VDJ recombination[14]. Consequently, RAG deficiency leads to severe combined immunodeficiency (SCID), a condition in which patients lack T cells and B cells, but not natural killer cells[14] (FIG. 1). Patients with SCID are particularly susceptible to opportunistic infections. Various forms of hypomorphic mutations in *RAG1* and *RAG2* have also been described[15,16]: depending on the level of

Table 1 | **Primary immunodeficiency syndromes that can have autoimmune manifestations**

Immune pathway	Gene(s)	Syndrome(s)
T cell development and tolerance	• *RAG1* and *RAG2* • *RAG1*, *RAG2* and *DCLRE1C* • *AIRE* • *FOXP3*	• SCID, CID and CVID • Omenn syndrome • APECED • IPEX and IPEX-like syndromes
T cell signalling	• *CTLA4* and *LRBA* • PI3K-related genes • *DOCK8* • *LAT* • JAK and STAT family genes	• CTLA4 insufficiency, CID and CVID • CVID and CID • DOCK8 syndrome • SCID and CID • CMC and hyper IgE syndrome
Interferon signalling pathway	• *TMEM173* • *ACP5*	• SAVI • SPENCD
Complement pathway	Complement protein genes	SLE
Resolution of inflammation	*FAS* and *FASL*	ALPS

ALPS, autoimmune lymphoproliferative syndrome; APECED, autoimmune–polyendocrinopathy–candidiasis–ectodermal dystrophy; CID, combined immunodeficiency; CMC, chronic mucocutaneous candidiasis; CTLA4, cytotoxic T lymphocyte protein 4; CVID, common variable immune deficiency; DOCK8, dedicator of cytokinesis protein 8; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; JAK, Janus kinase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; SAVI, STING-associated vasculopathy with onset in infancy; SCID, severe combined immunodeficiency; SLE, systemic lupus erythematosus; SPENCD, spondyloenchondrodysplasia; STAT, signal transducer and activator of transcription.

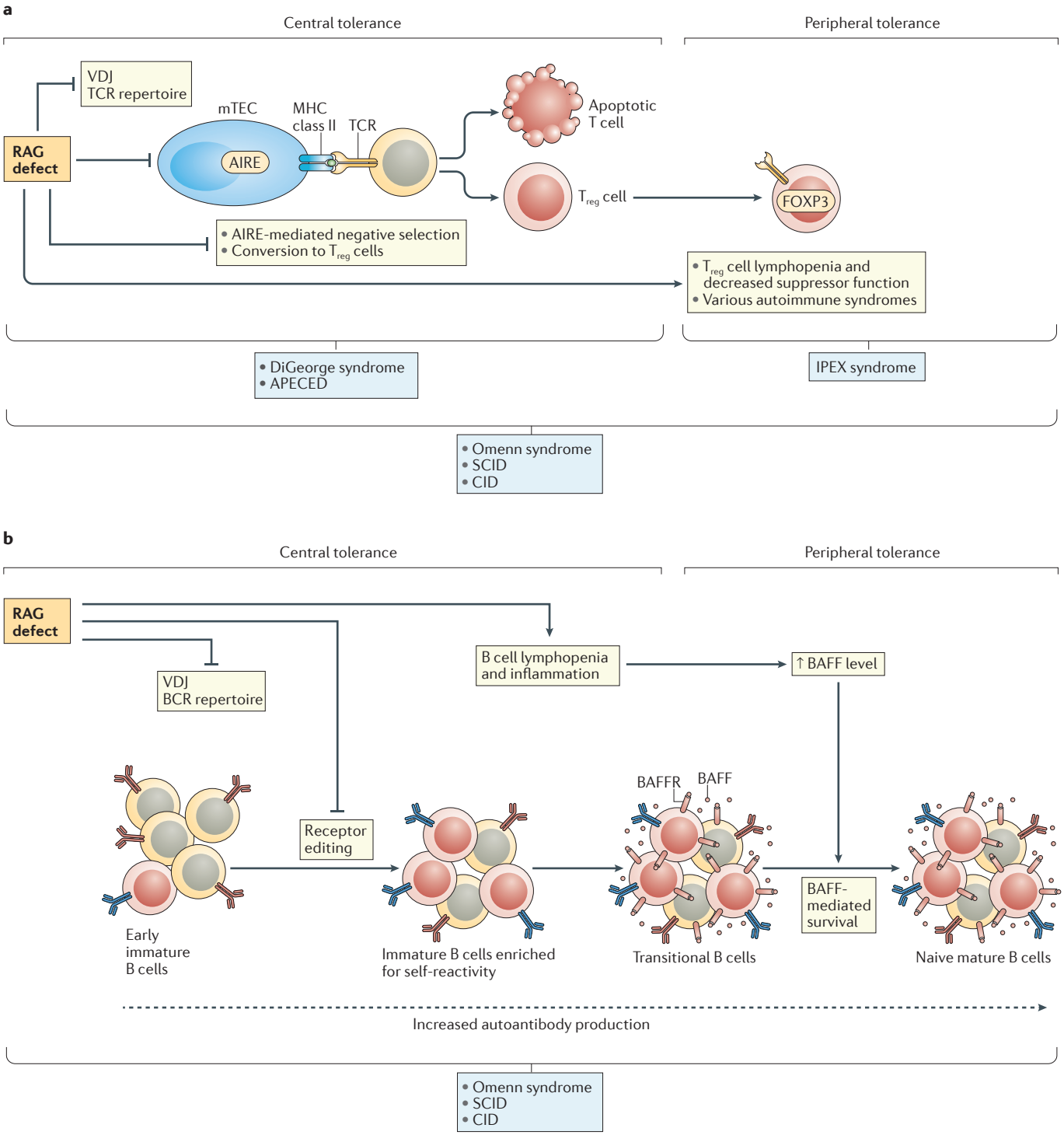

Figure 1 | **Defects in lymphocyte development and central and peripheral tolerance. a** | VDJ recombination-activating protein (RAG) defects and impaired VDJ recombination lead to a restricted T cell receptor (TCR) repertoire, defects in autoimmune regulator (AIRE) cause a loss of negative selection, and both of these deficiencies result in various autoimmune syndromes characterized by regulatory T (T_{reg}) cell lymphopenia or decreased T_{reg} cell suppressive function. Disturbances of embryogenesis in the third and fourth pharyngeal pouches lead to DiGeorge syndrome, whereas mutations in *AIRE* cause autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED). Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is the result of deficiency in the transcription factor FOXP3, which causes a reduction in the number of T_{reg} cells. CD4 lymphopenia is seen in all of these syndromes, as well as in the presence of mutations in *DCLRE1C*, and all of these manifestations can present as Omenn syndrome. **b** | RAG defects also lead to impaired VDJ recombination in B cells, thereby causing restrictions to the B cell receptor (BCR) repertoire. The consequences of RAG defects are B cell lymphopenia, inflammation, increased levels of B cell-activating factor (BAFF; also known as TNFSF13B) and immature B cells with a high degree of self-reactivity, which lead to increased levels of autoantibody production. CID, combined immunodeficiency; mTEC, medullary thymic epithelial cell; SCID, severe combined immunodeficiency.

residual enzyme activity, patients present with different levels of oligoclonality in their T cell and B cell repertoires. These patients might still have low numbers of normal T cells and B cells, but they will also have many autoreactive lymphocytes that can cause various forms of autoimmunity, such as idiopathic CD4 lymphopenia, common variable immunodeficiency (CVID), combined immunodeficiency (CID), IgA deficiency and Omenn syndrome[14]. Patients with hypomorphic mutations in *RAG1* and *RAG2* are also susceptible to chronic infections with viruses such as cytomegalovirus, Epstein–Barr virus or John Cunningham virus[17].

Hypomorphic *RAG* mutations are the most common cause of Omenn syndrome, a condition in which patients present with lymphadenopathy, hepatosplenomegaly, eosinophilia, infiltrates of oligoclonal and activated T cells in multiple organs and severe hypogammaglobulinaemia with increased levels of IgE[18]. These manifestations of Omenn syndrome are caused by autoreactive T cells in a manner similar to the manifestations of graft-versus-host disease. Mutations in *DCLRE1C*, which encodes artemis, another protein involved in VDJ recombination, can also cause Omenn syndrome[19]. An increased frequency of autoantibodies and impaired B cell lymphopoesis, along with a disturbed selection process for autoreactive B cells, have also been observed in patients with RAG deficiencies[20] (FIG. 1b). The broad range of serum autoantibodies seen in these patients is consistent with the immune manifestations they present with, which include alopecia, vitiligo, granulomas, myasthenia gravis, vasculitis and psoriasis[20].

Diagnostically, phenotypic analysis of lymphocytes and PCR analysis of TCR excision circles (TRECs) or κ-deleting recombination excision circles (KRECs) can be useful screening methods for the discovery of abnormalities in T cell and B cell development when used before genetic sequencing analysis is carried out[21]. Although the results of preclinical trials of gene therapy for RAG2 deficiency have been promising[14], haematopoietic stem cell transplantation (HSCT) remains the most successful therapy for RAG deficiency to date.

Central tolerance. Two syndromes display features of disturbances in thymic T cell development: DiGeorge syndrome and autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) (FIG. 1a). Patients with DiGeorge syndrome have heterozygous deletions on chromosome 22q11.2, causing the incomplete development of the third and fourth pharyngeal pouches and leading to symptoms such as hypoparathyroidism and cardiac and facial malformations[22]. Partial DiGeorge syndrome has a prevalence of 1 in 4,000 live births and results in a hypoplastic or atypically located thymus[23]. The immunodeficiency seen in patients with complete or partial DiGeorge syndrome is dependent on the number and functionality of T cells, and the observed phenotype can vary from a SCID-like lack of T cells to an almost normal complement of T cells. Depending on the severity of the T cell phenotype, different levels of susceptibility to infections can occur. Autoimmune syndromes (such as autoimmune hypothyroidism, RA, vitiligo, psoriasis and autoimmune cytopenias) are seen in 8.5–10% of patients with partial DiGeorge syndrome owing to a lack of deletion of autoreactive cells[22,23]. By contrast, APECED is a monogenic disorder caused by a variety of mutations in AIRE, a transcription factor that regulates central immune tolerance[24]. T cell selection is impaired in these patients owing to the lack of AIRE in cells of the thymic epithelium[24], which has a knock-on effect on the development of regulatory T (T_{reg}) cells, leading to decreased numbers of T_{reg} cells in these patients[25]. Patients with APECED usually present with autoimmune manifestations in their endocrine organs, including the parathyroid glands, adrenal glands, gonads and thyroid gland[26,27]. Of these manifestations, the most severe are hypoparathyroidism and adrenal insufficiency, which affect more than 90% of patients with APECED[27]. In addition, chronic mucocutaneous candidiasis is observed in more than 80% of patients with APECED[27]. The majority of patients with APECED also have autoantibodies against IL-17, IL-22, and IFNω[26,27]. Therapeutic approaches for DiGeorge syndrome are mostly experimental. HSCT has a poor rate of success in patients with severe SCID-like phenotypes; however, thymus tissue transplantation has been used to successfully treat patients with DiGeorge syndrome who have thymic deficiency[28]. APECED, however, is usually treated by hormone replacement and immunosuppression when necessary.

Peripheral tolerance. Peripheral tolerance is regulated by T_{reg} cells, which are characterized by the expression of the surface antigens CD3, CD4 and CD25, and by the expression of the transcription factor FOXP3. Monogenic mutations in patients with complex autoimmune syndromes demonstrate the importance of T_{reg} cells in the maintenance of human immune homeostasis, especially in the gut. In humans, the discovery of the importance of T_{reg} cells started with the identification of mutations in *FOXP3*, which is located on the X chromosome, in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome[29] (FIG. 1a). These patients have diarrhoea and early-onset organ-specific autoimmunity, which leads to the destruction of organs such as the endocrine glands (including the pancreas and the gut)[29]. In mice, mutations in *Foxp3* cause the 'scurfy' phenotype, which produces symptoms similar to IPEX[30]. Despite mutations in *FOXP3* only causing a lack of T_{reg} cells in patients with IPEX syndrome, this deficiency is sufficient to produce a multi-organ immune disease[29]. Adoptive T cell transfer experiments in mice have shown that a lack of T_{reg} cells particularly affects the immune homeostasis of the gut[29]. Epigenetic analysis of the *FOXP3* locus in patients with an IPEX-like syndrome demonstrated that reduced numbers of T_{reg} cells might lead to autoimmune manifestations, even when mutations in *FOXP3* are not evident[31]. In this study, half of the patients also presented with infections such as septicaemia, cytomegalovirus, upper airway infections and pneumonia[31]. However, T_{reg} cells not only need to be present in the tissues to prevent autoimmunity, but also need to function correctly. In one group of patients, impaired T_{reg} cell function was found

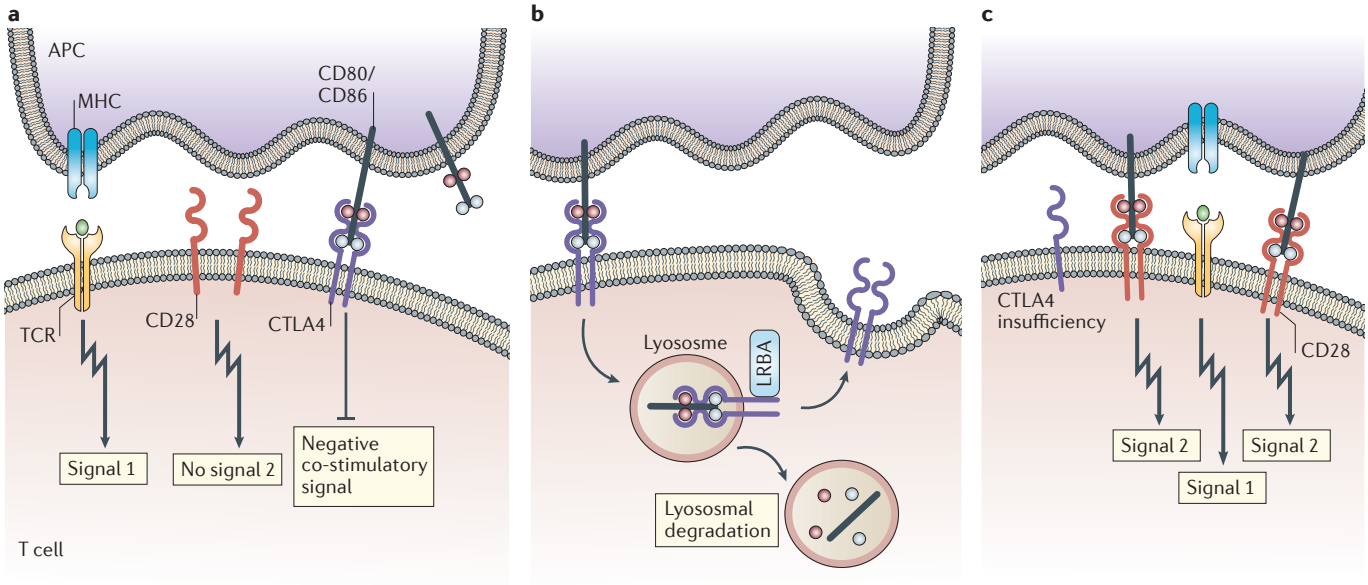

Figure 2 | **Defects in peripheral tolerance caused by mutations in co-stimulatory molecules. a** | CD28 delivers a co-stimulatory signal to T cells that is counteracted by cytotoxic T lymphocyte protein 4 (CTLA4), which binds with a higher affinity and avidity to the ligands shared by these two molecules, CD80 and CD86. **b** | By binding to the intra-cytoplasmic tail of CTLA4, lipopolysaccharide-responsive and beige-like anchor protein (LRBA) prevents CTLA4 degradation in the lysosome and helps CTLA4 to recirculate onto the surface of cells such as regulatory T cells. **c** | In patients with CTLA4 insufficiency, few CTLA4 molecules exist on the cell surface. The competition between CTLA4 and CD28 therefore swings in favour of CD28, leading to the activation of effector T cells. CTLA4 biology seems to be 'a sheer numbers game', hence, patients who lack LRBA have little CTLA4 on the surface of their T cells and display a phenotype similar to that seen in patients with CTLA4 insufficiency. APC, antigen-presenting cell; TCR, T cell receptor.

to be caused by CD25 deficiency[31]. The IL-2 receptor (CD25) is particularly important for the correct function of T_{reg} cells[32]. In light of the importance of T_{reg} cells in the prevention of autoimmune disorders, attempts have been made to restore T_{reg} cell numbers therapeutically. In patients with SLE, low-dose IL-2 therapy was beneficial for increasing the number of T_{reg} cells, and could potentially be of use in other autoimmune diseases[33].

T cell signalling

T cells are activated by two important signals: the first stimulatory signal reaches the T cell via TCR engagement with antigen presented on HLA molecules, and the second signal (known as the co-stimulatory signal) is provided by the co-stimulatory molecules CD28 and inducible T cell co-stimulator (ICOS). This co-stimulatory signal is finely tuned by two additional transmembrane receptors, cytotoxic T lymphocyte protein 4 (CTLA4) and programmed cell death protein 1 (PD1), the expression of which are upregulated at the T cell surface upon T cell activation. The ligation of these inhibitory receptors to their respective ligands leads to the downregulation of T cell activation[34]. Interference with this firmly regulated mechanism unbalances T cell homeostasis and can lead to immune-mediated diseases. Not surprisingly, perturbations that lead to increased T cell activation cause autoimmunity, whereas perturbations that lead to T cell anergy predispose individuals to recurrent and severe opportunistic infections and to tumour development[34].

Abnormal immune regulation. CTLA4 is needed to downregulate T cell activation as it binds with a higher affinity and avidity to the ligands CD80 and CD86 than CD28, thereby out-competing CD28 for binding partners and counter-balancing the co-stimulatory signal CD28 provides (FIG. 2a). Following binding, CTLA4 rips CD80 and CD86 out of the membrane of antigen presenting cells and 'eats' them in a process known as transendocytosis, thereby effectively rendering antigen-presenting cells devoid of co-stimulatory capacity[35] (FIG. 2b). Transendocytosis is dependent on the presence of sufficient CTLA4 molecules at the surface of T_{reg} cells; most CTLA4 molecules are stored in vesicles in the cytoplasm and transported to the surface of T_{reg} cells upon activation. Not only is the capacity to bind CD80 and CD86 important to ensure correct CTLA4 biology, but so is the correct pairing of CTLA4 dimers and their intracellular shuttling. Heterozygous mutations in CTLA4 that affect either ligand binding, homodimerization of CTLA4 or shuttling to the cell surface lead to complex autoimmune conditions known in humans as CTLA4 insufficiency[36,37] (FIG. 2c). In mice, the homozygous deletion of *Ctla4* causes severe autoimmunity and eventual death[38]. Moreover, biallelic mutations in the gene encoding lipopolysaccharide-responsive and beige-like anchor protein (LRBA), a protein that binds to the cytoplasmic tail of CTLA4 and prevents its degradation in lysosomes (FIG. 2b), cause a similar phenotype to that seen in patients with CTLA4 insufficiency. The phenotypes of CTLA4 insufficiency and LRBA deficiency are

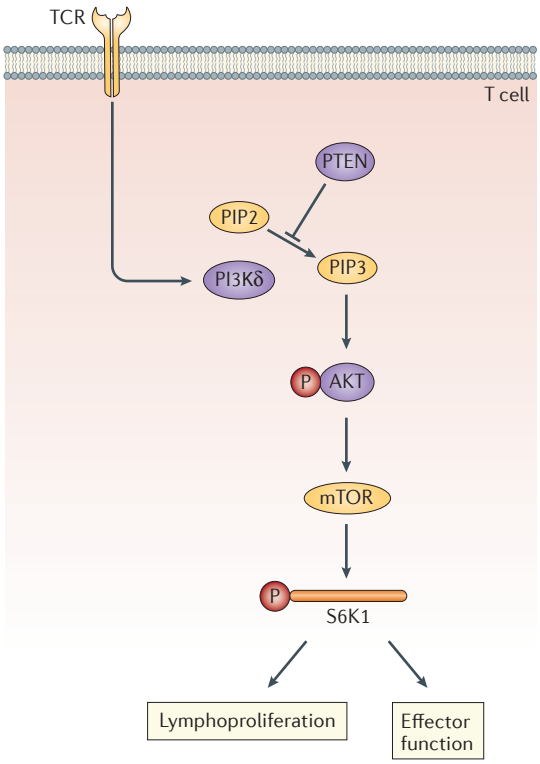

Figure 3 | **Defects in the T cell receptor signalling pathway.** Constitutive activation of phosphatidylinositol 4,5-bisphosphate 3-kinase δ (PI3Kδ) by gain-of-function mutations leads to an increase in RACα serine/threonine-protein kinase (AKT) phosphorylation and ribosomal protein S6 kinase β1 (S6K1) signalling. Loss of the PI3K inhibitor PTEN likewise leads to increased PI3Kδ activity, causing lymphoproliferation, autoimmunity and antibody deficiency. mTOR, mechanistic target of rapamycin; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; TCR, T cell receptor.

characterized by the infiltration of organs by activated effector T cells and by the occurrence of autoimmune cytopenias[39,40]. These observations in patients with primary immunodeficiency syndromes demonstrate the importance of CTLA4–LRBA in the maintenance of immune homeostasis specifically, and for T_{reg} cells in general. CTLA4 signalling has effectively been exploited by two CTLA4-Fc fusion proteins, abatacept and belatacept, which are licensed for use in the treatment of RA and T cell-mediated graft rejection following kidney transplantation, respectively. Both molecules could now be investigated in clinical trials for use in CTLA4 insufficiency and LRBA deficiency. The efficacy of hydroxychloroquine as a therapy in SLE and other rheumatic diseases is also thought to be attributable to effects on CTLA4 expression[41].

Abnormal immune activation. The study of primary immunodeficiency syndromes has led to the identification of several monogenic defects that cause constitutive T cell activation[42], the paramount example being activating mutations in *PIK3CD*, which encodes phosphatidylinositol 4,5-bisphosphate 3-kinase subunit δ (PI3Kδ), a protein that is selectively expressed in leukocytes. Increased PI3Kδ signalling in lymphocytes leads to increased phosphorylation of RACα serine/threonine-protein kinase (AKT; also known as protein kinase B), activation of mechanistic target of rapamycin (mTOR) and phosphorylation of ribosomal protein S6 kinase β1, which drives T cells to proliferate and develop effector function[43] (FIG. 3). Likewise, mutations that impair the function of proteins that normally inhibit the PI3K signalling pathway (such as PI3K regulatory subunit α or the negative regulator of PI3K signalling, PTEN) lead to an over-activation of this signalling pathway with the same consequences as activating mutations in *PIK3CD*: increased T cell proliferation, immune activation and prolonged effector function[44]. A PI3Kδ inhibitor is currently under investigation for use in patients with activating mutations in *PIK3CD* (REF. 45).

As indicated, patients with such a constitutive increase in T cell activation often develop systemic autoimmunity, which most frequently affects the gut, the lungs and the haematopoietic system (in the form of autoimmune cytopenia)[36]. There is typically a pronounced T cell infiltrate in affected organs (FIG. 4) that is capable of the destruction of the whole organ, leading to organ failure and death[36]. Interestingly, however, not all patients display the same phenotype, although a reduced penetrance and a highly variable expressivity seem to be characteristic, especially in the autosomal-dominant forms of these conditions[46]. Whether a second genetic mutation, epigenetic changes or environmental factors (for example, infections) influence the penetrance and expressivity of such autoimmunity is currently being investigated. In general, these monogenic conditions teach us that over-activation of the T cell compartment can lead to autoimmunity, explaining why selective mTOR inhibitors (such as sirolimus) and the inhibition of co-stimulatory signals (or the support of signals that inhibit T cell activation) might be ideal targeted treatments for autoimmune diseases.

TCR signalling. Patients with CID with autosomal recessive dedicator of cytokinesis protein 8 (DOCK8) deficiency were first described in 2009 (REF. 47). The importance of DOCK8 in immune function comes from its central role in the regulation of the actin cytoskeleton, as well as in signal transducer and activator of transcription 3 (STAT3) activation. Deficiencies in DOCK8 lead to dysfunction in numerous cellular processes, such as cell polarization and migration, cell adhesion and immune synapse formation, regulation of STAT3, phosphorylation and translocation of STAT3 to the nucleus, cytolytic granule release, actin cytoskeleton organization and dysfunction of T_{reg} cell suppressive function[47]. Clinically, the consequences of DOCK8 deficiency are recurrent infections, allergic diseases (including eczema and allergies), autoimmunity and virally driven malignancies[48].

Mutations in linker for activation of T cells (LAT) have also been observed in patients with SCID[49], as well as in patients with CID who have autoimmune manifestations[50]. In both sets of patients, homozygous mutations

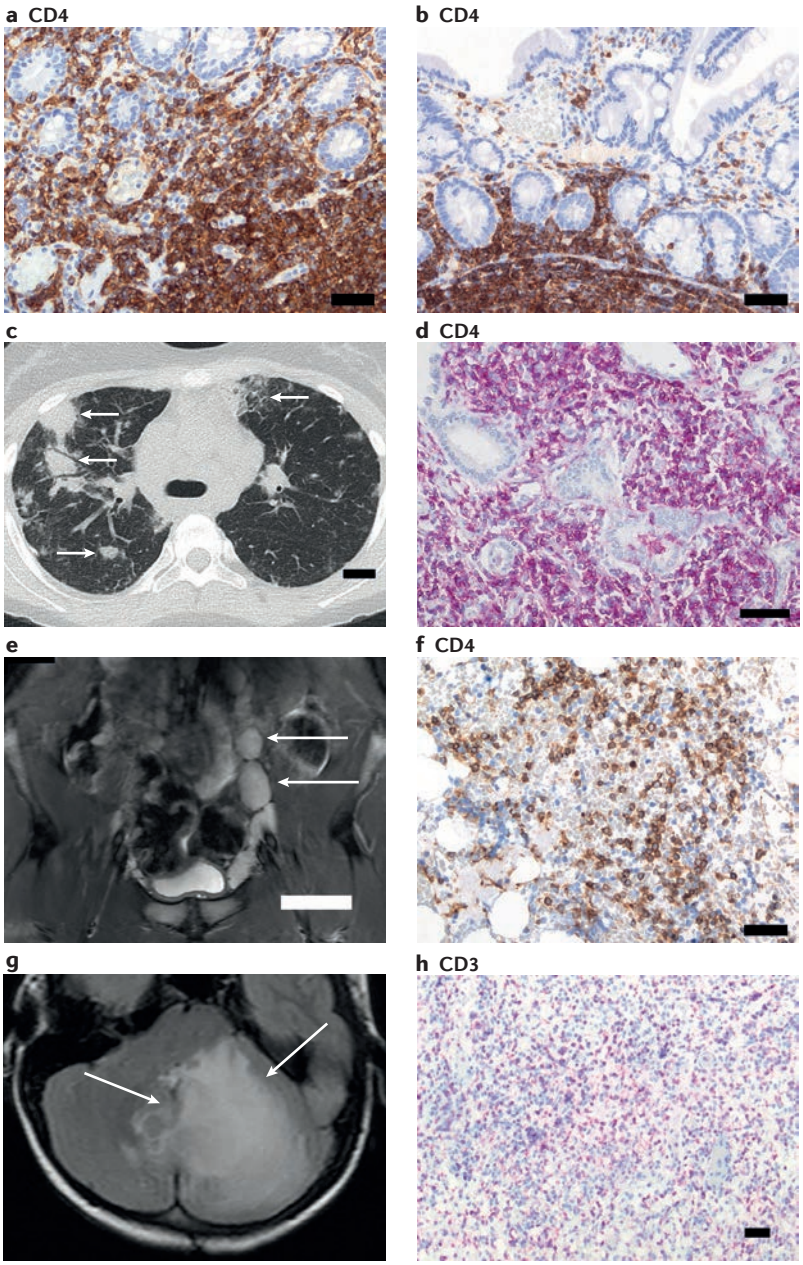

Figure 4 | **Tissue infiltration and lymphadenopathy in patients with *CTLA4* mutations. a,b** | Duodenal tissue stained for CD4. **c** | High-resolution chest CT scan of the lungs (arrows indicate granulomatous-lymphocytic infiltrations). **d** | Pulmonary tissue showing lymphoid fibrotic lesions stained for CD4. **e** | MRI scan of lymphocytic proliferation in the pelvic area (arrows indicate enlarged lymph nodes). **f** | CD4 staining of bone marrow tissue. **g** | Gadolinium-enhanced MRI scan of the cerebellum (arrows indicate lesions). **h** | Tissue from a resected cerebellar lesion stained for CD3. Reproduced from Schubert, D. *et al*. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat. Med.* **20**, 1410–6 (2014).

in exon 5, a premature stop codon and protein truncation leading to complete LOF were observed. All three patients with CID were affected with early-onset autoimmune manifestations and had lymphocyte counts and antibody levels that were initially normal. As the disease progressed, lymphocytopenia and opportunistic infections were observed[50].

JAK–STAT signalling pathway. Janus kinases (JAKs) were first described in 1989 (REF. 51). Following the binding of various cytokines (including type I IFNs, type II IFNs, granulocyte-macrophage colony-stimulating factor, several interleukins, erythropoietin, growth hormone and prolactin) to their respective receptors, JAKs are phosphorylated and, in turn, phosphorylate various members of the STAT family of transcription factors[52] (FIG. 5). Members of the JAK family associate with the proline-rich membrane-proximal box 1/box 2 domain of a variety of cytokine receptors. Each cytokine receptor preferentially activates certain JAKs; for example, the IL-6 receptor activates JAK1, JAK2 and TYK2, whereas the IL-2 receptor γ chain preferentially activates JAK1 and JAK3 (REFS 53,54). Once a JAK has been phosphorylated, it recruits specific STATs via its SH2 domain, which become JAK substrates. Phosphorylated STATs are released from the receptor, dimerize and translocate to the nucleus, where they bind to specific enhancer elements and initiate gene transcription (FIG. 5).

The mammalian STAT family contains seven members, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. To a certain degree, specific JAKs preferentially bind to specific STATs; however, most cytokines will activate several STATs[54]. Furthermore, many genes are regulated by several STAT molecules; therefore, a large amount of overlap and pleiotropy exists in the gene activation profile of different cytokines[55]. The JAK–STAT pathway has gained importance for rheumatologists since the development of JAK inhibitors that therapeutically target cytokine signalling pathways in rheumatic diseases. These JAK inhibitors are now widely used, especially in the fields of rheumatology and oncology. Importantly, different JAK inhibitors will target different JAKs, so the cytokines inhibited by each inhibitor might differ.

Mutations in JAK and STAT molecules are associated with many diseases in humans. In 2017, a GOF mutation in JAK1 was described that caused atopic dermatitis, eosinophila, hepatosplenomegaly and autoimmune thyroid disease[56]. Biallelic mutations in JAK3 have long been known to cause SCID owing to the lack of crucial cytokine signalling in T cells[57]. By contrast, a GOF mutation in JAK2 (Val617Phe) leads to polycythaemia vera and other myeloproliferative diseases[58,59], owing not only to the involvement of JAK2 in cytokine signalling pathways, but also to its involvement in the erythropoietin and thrombopoietin signalling pathways. Mutations in STATs can either decrease STAT signalling (LOF mutations), or increase STAT signalling (GOF mutations) (FIG. 5).

STAT1 GOF mutations are heterozygous and impair the development of T helper 17 (T_H17) cells by causing a relative decrease in STAT3 signalling[60]. The lack of T_H17 cells leads to a lack of IL-17 and IL-22, two cytokines that are crucially important for the amplification of myeloid-derived danger signals against *Candida* spp. and staphylococci, especially in the skin and mucosa. Patients with STAT1 GOF mutations are therefore susceptible to chronic mucocutaneous candidiasis and often develop folliculitis and skin abscesses[61]. By contrast, IFNα signalling is increased in patients with these GOF mutations:

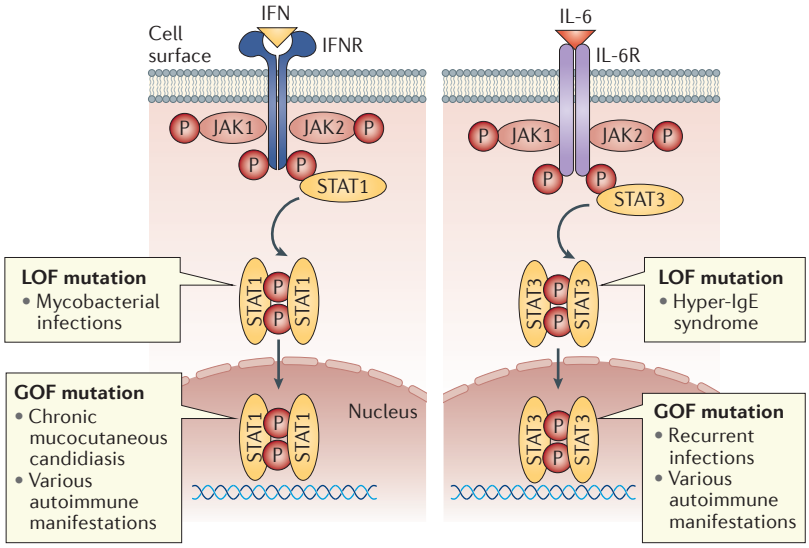

Figure 5 | **Defects in the JAK–STAT signalling pathway.** The binding of various cytokines to their respective receptors stimulates the phosphorylation of the intracellular domains of these receptors by various members of the Janus kinase (JAK) family. Subsequently, members of the signal transducer and activator of transcription (STAT) family are phosphorylated, form dimers and translocate to the nucleus, where they bind to specific enhancer elements and cause transcription of specific genes. GOF, gain-of-function; IFN, interferon; IFNR, interferon receptor; IL-6R, IL-6 receptor; LOF, loss-of-function.

patients often have autoimmune manifestations such as hypothyroidism, autoimmune hepatitis, type I diabetes mellitus, idiopathic thrombocytopenic purpura and autoimmune haemolytic anaemia[62]. In the autosomal recessive disorder caused by STAT1 LOF mutations, T_H1 cell differentiation is impaired, thereby reducing both type I and type II IFN production[63]. Patients with STAT1 LOF mutations are characterized by a predisposition to mycobacterial and viral infections[64], although heterozygous STAT1 LOF mutations have been described in which only the anti-mycobacterial defence system is impaired[65].

STAT3 GOF mutations cause a lymphoproliferative disorder termed large granular lymphocytosis, a form of indolent lymphoma[66]. Heterozygous germline GOF STAT3 mutations have also been observed and were associated with lymphoproliferation and early-onset autoimmune manifestations such as autoimmune cytopenia, lung, gastrointestinal or hepatic involvement and polyarthritis[67]. STAT3 LOF mutations (also termed STAT3 dominant-negative mutations) cause a phenotype called hyper IgE syndrome (alternatively known as Job syndrome or Buckley syndrome), which is characterized by recurrent skin boils, pneumonia and high levels of IgE[68]. As with STAT1 GOF mutations, STAT3 LOF mutations also lead to a paucity of T_H17 cells, rendering the affected individual susceptible to infection with staphylococci and *Candida* spp[69].

The association between LOF mutations in JAK and STAT molecules with viral infections should be kept in mind when JAK inhibitors are used to treat patients with rheumatic diseases. Unlike a genetic mutation, JAK inhibitors do not block cytokine signalling completely, hence the rate of infections observed in studies is generally low; however, there does seem to be an increased rate of herpes zoster infection after initiation of JAK inhibitors[70]. In addition, IL-17 blockade is currently used as a therapy for spondyloarthritis: the rate of infections in patients receiving this therapy is not large, but a higher rate of candidiasis has been described, as predicted on the basis of experiences with patients who lack T_H17 cells[71].

Interferon signalling pathway

Endogenous DNA and RNA molecules can induce an inflammatory cascade via the endosomal Toll-like receptors (TLR3, TLR7, TLR8 and TLR9), cytosolic RIG-I-like helicases or the specialized DNA sensor cGAS. These pathways all lead to the induction of type I IFNs following the activation of IFN regulatory factors[72]. However, interferonopathies that lead to autoimmunity and immunodeficiencies are not only caused by nuclease defects; autoimmunity is also observed in situations in which there is consecutive activation and/or enhanced sensitivity of an innate immune sensor or an adaptive receptor. Moreover, a defect in negative regulators of type I IFNs can lead to this clinical picture[73].

Type I IFNs are the most important cytokines in the pathogenesis of SLE[74]. In addition to the known interferon-related genetic risk factors for SLE, a number of interferonopathies have been discovered; for example, mutations in *TMEM173* (encoding stimulator of interferon genes protein (STING)) confer a GOF in STING activity[73]. Such mutations lead to either constitutive or environmental stimulus-dependent induction of type I IFNs, causing diseases such as STING-associated vasculopathy with onset in infancy (SAVI), which is associated with systemic inflammation, increased levels of C-reactive protein and severe vasculopathy[75,76]. Spondyloenchondrodysplasia (SPENCD), another interferonopathy, is associated with hypomorphic mutations in *ACP5* (which encodes tartrate-resistant acid phosphatase type 5, a marker of osteoclast differentiation) that lead to impaired endochondral bone growth and a type I IFN signature[77].

Immune complexes and apoptotic debris

The clearance of immune complexes is mediated by the binding of cellular debris to either complement proteins, natural antibodies (mostly IgM) or specific IgG antibodies and the subsequent uptake of these complexes by cells in the liver and spleen. Among the complement proteins, immune complexes are mostly bound by complement protein C3b, which then binds to complement receptor 1 on erythrocytes. These cells then transport the bound immune complexes to the liver and spleen, where the complexes are phagocytosed (FIG. 6a).

Deficiencies in components of the early part of the complement pathway, such as complement proteins C1q, C1r, C1s, C2 and C4, are associated with low levels of C3b and contribute to impaired clearance of immune complexes[78]. In such circumstances, the prolonged presence of cellular material in the blood might induce the formation of autoantibodies, and immune complexes themselves can further stimulate autoimmune responses.

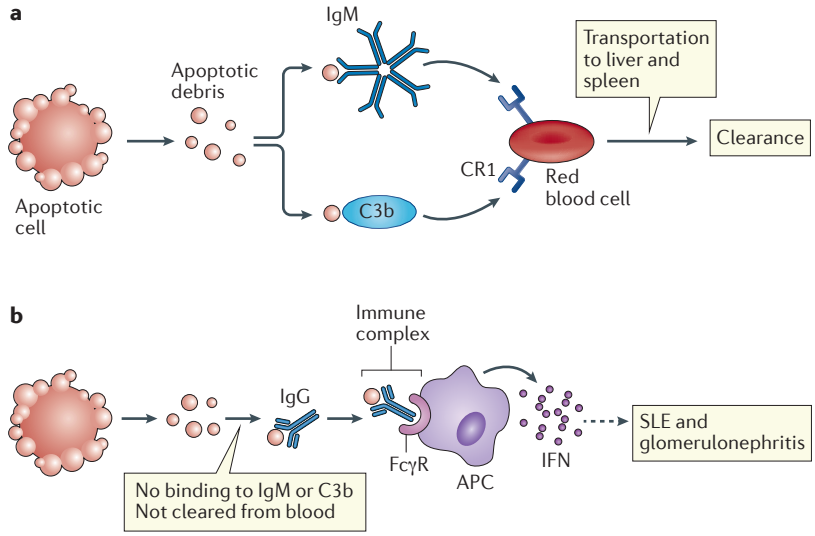

Figure 6 | **Defects in the clearance of apoptotic debris and immune complexes.** **a** | Cellular apoptotic debris is normally cleared through binding to complement protein C3b or to naturally occurring IgM antibodies. Immune complexes consisting of C3b or IgM and debris bind to complement receptor 1 (CR1) on erythrocytes and are transported to the liver and spleen where they are phagocytosed and removed from the circulation. **b** | In patients with complement deficiencies or selective IgM deficiency, cellular debris is not removed from the circulation, but instead stimulates the production of IgG autoantibodies. Immune complexes of IgG and debris bind to Fc receptors on macrophages and dendritic cells, inducing the production of type I interferon (IFN) and other cytokines. In particular, increased type I IFN production can lead to the development of systemic lupus erythematosus (SLE). APC, antigen-presenting cell; FcγR, Fcγ receptor.

Accordingly, complexes of Sjögren syndrome-related antigen A (SSA; also known as Ro) and anti-SSA antibodies stimulate the production of TNF by macrophages by crosslinking TLRs and Fc receptors[79], and complexes of U1 small nuclear ribonucleoprotein (U1snRNP) and anti-U1snRNP antibodies (found in patients with SLE) induce the production of IFNα by plasmacytoid dendritic cells[80]. Furthermore, immune complexes of double-stranded DNA (dsDNA) or nucleosomes and anti-dsDNA antibodies might be able to induce glomerulonephritis in patients with SLE, since such immune complexes are deposited on the glomerular basement membrane[81] (FIG. 6b). By contrast, natural IgM antibodies might be protective against damage from some autoantigens: the injection of IgM anti-dsDNA antibodies into lupus-prone NZB×NZW F1 mice prevented the onset of glomerulonephritis[82]. In this model, complexes of IgM and dsDNA were cleared through the liver, so pathogenic complexes of IgG and dsDNA were not able to form[82].

Complement pathway

Despite being rare, defects in components of the early part of the complement pathway are strongly associated with SLE, which occurs in 80% of individuals with C1 or C4 deficiency, and in 30% of those with C2 deficiency[83]. C2 deficiency, which occurs in Europe at a prevalence of between 1:10,000 and 1:20,000, is a primary immunodeficiency syndrome that is also associated with upper respiratory tract infections in children[84]. Complete deficiency of C3 results in susceptibility to severe bacterial infections (in particular from *Pneumococcus* spp. and *Haemophilus influenza*) in early childhood[85]. Children who survive these early infections will usually subsequently develop immune complex-mediated autoimmune manifestations such as glomerulonephritis[86]. Notably, heterozygous C3 deficiency, which is associated with reduced serum concentrations of C3, does not produce clinically relevant manifestations. Deficiencies in the components of the membrane attack complex (C5–C9) predispose individuals to infections with *Neisseria* spp.[87]; however, since these complement proteins are not involved in the clearance of immune complexes, these deficiencies are not associated with autoimmunity.

A complete deficiency of complement receptor 3 (CR3; also known as CD11b), an integrin that binds to inactivated C3b, is rare and is associated with severe bacterial infections and, in one case report, with SLE[88]. However, the functional Arg77His variant of CR3 causes impaired phagocytosis of inactivated C3b-coated erythrocytes and is associated with SLE[89,90]. The response of phagocytes to IgG-containing immune complexes is further influenced by the interaction of such immune complexes with Fcγ receptors (FIG. 6b). Fcγ receptor 3 (FcγRIII; also known as CD16) and FcγRI (also known as CD64) are activating receptors, whereas FcγRIIb (also known as CD32b) is inhibitory[91]. The Ile232Thr variant of FcγRIIb abolishes the normal inhibitory activity of this receptor on macrophages and B cells and is associated with SLE, and interestingly also provides protection from malaria[92]. By contrast, copy number variants exist in *FCGR3B*, which encodes FcγRIIIb: low copy numbers are thought to interfere with the clearance of immune complexes by phagocytes and are associated with SLE[93]. In addition, a polymorphism in *FCGR3A*, which encodes FcγRIIIa, a protein that is expressed on monocytes and natural killer cells, is associated with SLE[94]. Currently, new therapeutic approaches with soluble Fcγ receptor constructs are under investigation, and should be able to block the interaction between immune complexes and Fc receptors[95].

Resolution of inflammation

Cells of the immune system that proliferate in response to an infection should, in theory, reduce in number once the threat of infection has passed so as to avoid overactivation of the immune system and autoimmunity. Two major mechanisms contribute to the death of T cells: activation-induced cell death (AICD), which mostly occurs in activated T cells that are re-stimulated via the TCR, and activated T cell autonomous death (ACAD), which occurs independently of TCR re-stimulation. AICD is influenced by many factors, such as the cytokines IL-2, IL-4 and IFNγ and proteins with anti-apoptotic function such as apoptosis regulator BCL2 or BCL2-associated agonist of cell death; however, the TNF receptor superfamily member 6 (also known as Fas) signalling pathway is the most important mechanism of AICD[96]. The expression of Fas on activated B cells and T cells and of Fas ligand (FasL; also known as TNF ligand superfamily member 6) on activated T cells is increased, and interaction between Fas and FasL triggers the caspase cascade, leading to apoptosis[97] (FIG. 7).

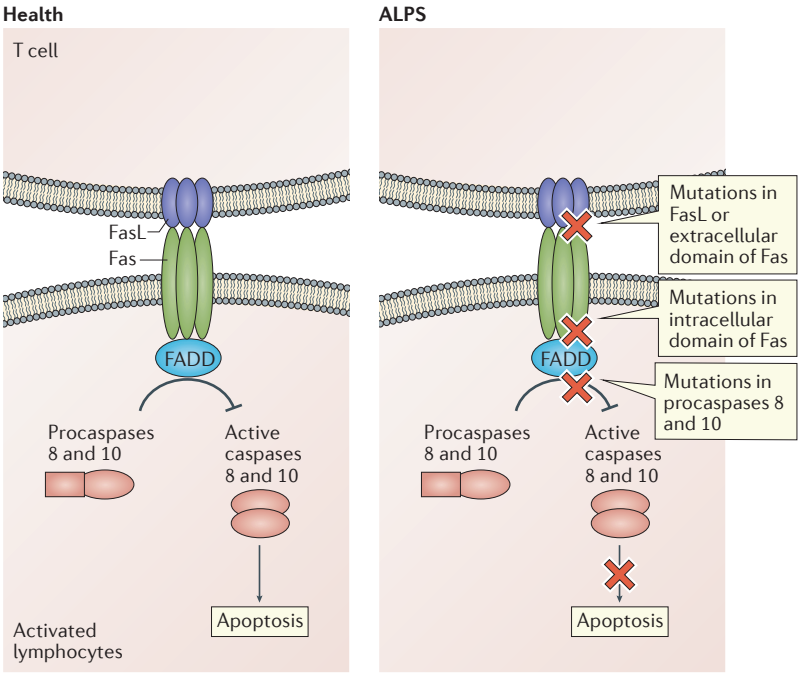

Figure 7 | **Defects in apoptosis.** Binding of Fas ligand (FasL; also known as TNF ligand superfamily member 6) to the extracellular domain of Fas (also known as TNF receptor superfamily member 6) leads to the formation of the death-inducing signalling complex, which is composed of 5–7 molecules of Fas and 5 molecules of Fas-associated death domain protein (FADD). FADD then interacts with procaspases 8 and 10, transforming them into active caspases. Active caspases 8 and 10 subsequently activate other procaspases, in particular procaspase 3, and initiate a proteolytic pathway that culminates in apoptosis. In patients with autoimmune lymphoproliferative syndrome (ALPS), genetic defects in any of these molecules can interfere with the apoptosis of lymphocytes, causing lymphocytes to accumulate and cause disease.

Autoimmune lymphoproliferative syndrome (ALPS) is caused by a defect in apoptosis and is characterized by the expansion of lymphocytes, mainly $CD3^+CD4^-CD8^-$ T cells, and by polyclonal hypergammaglobulinemia[98]. Patients with ALPS develop splenomegaly, lymphadenopathy and, frequently, autoimmune cytopenia and autoimmune organ disease, but also have an increased risk of developing cancer, particularly lymphoma[98]. The majority of patients with ALPS carry heterozygous germline or somatic mutations in *FAS*, and a few patients have mutations in *FASLG*[98–101] (FIG. 7). Whereas lupus-prone MRL/lpr mice carrying *Fas* mutations develop lupus-like disease with anti-nuclear and anti-dsDNA antibodies (the lymphoproliferation disorder in mice is explained by defects in Fas that affect apoptosis)[102], humans with *FAS* mutations do not usually have antibodies against dsDNA[101]. In addition, sirolimus, an mTOR inhibitor, is effective at normalizing the number of expanded $CD3^+CD4^-CD8^-$ T cells in patients with ALPS, as well as at reducing the clinical manifestations[103].

SLE, arthritis and other autoimmune diseases are often associated with chronic granulomatous disease, which is caused by a defect in the production of reactive oxygen species by phagocytes and leads to an inability to kill a wide variety of pathogens[104]. In these patients, mutations in components of the NADPH oxidase complex lead to decreased production of reactive oxygen species (which would normally inhibit autophagy and T cell activation by phagocytes) and induce a state of hyperinflammation[105].

Conclusions

In the future, genetic analyses of both autoimmune diseases and primary immunodeficiency syndromes are likely to reveal more genes that are involved in the pathogenesis of both groups of diseases. This knowledge will enable clinicians to intervene more specifically and to treat these diseases more successfully than they are currently able to. In addition, knowledge of the consequences of genetic alterations in primary immunodeficiencies will help researchers to predict potential adverse events related to new treatments for autoimmune diseases, such as the increased risk of candidiasis during treatment with anti-IL-17 antibodies or the risk of viral infections when using anti-interferon therapies.

Overall, this Review should help to familiarize rheumatologists with the issues surrounding primary immunodeficiencies that can present as autoimmune diseases. For a long time, it has been known that patients with autoimmune diseases such as SLE and RA present with disturbances in their immune system. Although the research community has learned much about the genetic mutations that are associated with these diseases, in the future, polymorphisms are likely to be discovered that will clarify the origin of some rheumatic diseases that are currently unexplained. A better understanding of the underlying mechanisms of immunodeficiency and immune dysregulation will enable the subsequent development of individualized therapies, some of which are already available (for instance therapies for CTLA4 or LRBA deficiency) or are currently under investigation (such as therapies for PI3K deficiencies). In addition, a good understanding of primary immunodeficiency syndromes is helpful for the evaluation of infection-related adverse events caused by DMARDs when treating rheumatic diseases.

1. Fischer, A., Provot, J. & Jais, J. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. *J. Allergy Clin. Immunol.* **140**, 1388–1393.e8 (2017).
2. Okada, Y. *et al.* Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* **506**, 376–381 (2014).
3. Grimbacher, B., Warnatz, K., Yong, P. F. K., Korganow, A.-S. & Peter, H.-H. The crossroads of autoimmunity and immunodeficiency: lessons from polygenic traits and monogenic defects. *J. Allergy Clin. Immunol.* **137**, 3–17 (2016).
4. Carroll, M. C. & Isenman, D. E. Regulation of humoral immunity by complement. *Immunity* **37**, 199–207 (2012).
5. Ghodke-Puranik, Y. & Niewold, T. B. Immunogenetics of systemic lupus erythematosus: a comprehensive review. *J. Autoimmun.* **64**, 125–136 (2015).
6. Deng, Y. & Tsao, B. P. Advances in lupus genetics and epigenetics. *Curr. Opin. Rheumatol.* **26**, 1–11 (2014).
7. Rodero, M. P. & Crow, Y. J. Type I interferon-mediated monogenic autoinflammation: the type I interferono-pathies, a conceptual overview. *J. Exp. Med.* **213**, 2527–2538 (2016).
8. Rönnblom, L. The importance of the type I interferon system in autoimmunity. *Clin. Exp. Rheumatol.* **34**, 21–24 (2016).
9. Elkon, K. B. & Stone, V. V. Type I interferon and systemic lupus erythematosus. *J. Interferon Cytokine Res.* **31**, 803–812 (2011).
10. Lee-Kirsch, M. A. *et al.* Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nat. Genet.* **39**, 1065–1067 (2007).
11. Lee-Kirsch, M. A., Wolf, C. & Günther, C. Aicardi-Goutières syndrome: a model disease for systemic autoimmunity. *Clin. Exp. Immunol.* **175**, 17–24 (2014).

12. Molineros, J. E. *et al.* Admixture mapping in lupus identifies multiple functional variants within IFIH1 associated with apoptosis, inflammation, and autoantibody production. *PLoS Genet.* **9**, e1003222 (2013).
13. Gao, D. *et al.* Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. *Proc. Natl Acad. Sci. USA* **112**, E5699–E5705 (2015).
14. Notarangelo, L. D., Kim, M.-S., Walter, J. E. & Lee, Y. N. Human RAG mutations: biochemistry and clinical implications. *Nat. Rev. Immunol.* **16**, 234–246 (2016).
15. Villa, A., Marrella, V., Rucci, F. & Notarangelo, L. D. Genetically determined lymphopenia and autoimmune manifestations. *Curr. Opin. Immunol.* **20**, 318–324 (2008).
16. Schröder, C. *et al.* Evaluation of RAG1 mutations in an adult with combined immunodeficiency and progressive multifocal leukoencephalopathy. *Clin. Immunol.* **179**, 1–7 (2016).
17. Buchbinder, D. *et al.* Identification of patients with RAG mutations previously diagnosed with common variable immunodeficiency disorders. *J. Clin. Immunol.* **35**, 119–124 (2015).
18. Henderson, L. A. *et al.* Expanding the spectrum of recombination-activating gene 1 deficiency: a family with early-onset autoimmunity. *J. Allergy Clin. Immunol.* **132**, 969–971.e2 (2013).
19. Lee, P. P. *et al.* The many faces of Artemis-deficient combined immunodeficiency — two patients with DCLRE1C mutations and a systematic literature review of genotype-phenotype correlation. *Clin. Immunol.* **149**, 464–474 (2013).
20. Walter, J. E. *et al.* Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency. *J. Clin. Invest.* **125**, 4135–4148 (2015).
21. Atschekzei, F., Ahmad, F., Witte, T., Jacobs, R. & Schmidt, R. E. Limitation of simultaneous analysis of T-cell receptor and κ-deleting recombination excision circles based on multiplex real-time polymerase chain reaction in common variable immunodeficiency patients. *Int. Arch. Allergy Immunol.* **171**, 136–140 (2016).
22. Gennery, A. R. *et al.* Antibody deficiency and autoimmunity in 22q11.2 deletion syndrome. *Arch. Dis. Child.* **86**, 422–425 (2002).
23. Tison, B. E. *et al.* Autoimmunity in a cohort of 130 pediatric patients with partial DiGeorge syndrome. *J. Allergy Clin. Immunol.* **128**, 1115–1117.e3 (2011).
24. Hinterberger, M. *et al.* Autonomous role of medullary thymic epithelial cells in central CD4$^+$ T cell tolerance. *Nat. Immunol.* **11**, 512–519 (2010).
25. Cavadini, P. *et al.* AIRE deficiency in thymus of 2 patients with Omenn syndrome. *J. Clin. Invest.* **115**, 728–732 (2005).
26. De Martino, L. *et al.* APECED: a paradigm of complex interactions between genetic background and susceptibility factors. *Front. Immunol.* **4**, 331 (2013).
27. Ferre, E. M. N. *et al.* Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight* **1**, e88782 (2016).
28. Davies, E. G. *et al.* Thymus transplantation for complete DiGeorge syndrome: European experience. *J. Allergy Clin. Immunol.* http://dx.doi.org/10.1016/j.jaci.2017.03.020 (2017).
29. Bennett, C. L. *et al.* The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of *FOXP3*. *Nat. Genet.* **27**, 20–21 (2001).
30. Brunkow, M. E. *et al.* Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder. *Nat. Genet.* **27**, 68–73 (2001).
31. Barzaghi, F. *et al.* Demethylation analysis of the *FOXP3* locus shows quantitative defects of regulatory T cells in IPEX-like syndrome. *J. Autoimmun.* **38**, 49–58 (2012).
32. Barron, L. *et al.* Cutting edge: mechanisms of IL-2-dependent maintenance of functional regulatory T cells. *J. Immunol.* **185**, 6426–6430 (2010).
33. Von Spee-Mayer, C. *et al.* Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **75**, 1407–1415 (2016).
34. Friedline, R. H. *et al.* CD4$^+$ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. *J. Exp. Med.* **206**, 421–434 (2009).
35. Wing, K. *et al.* CTLA-4 control over Foxp3$^+$ regulatory T cell function. *Science* **322**, 271–276 (2008).
36. Schubert, D. *et al.* Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat. Med.* **20**, 1410–1416 (2014).
37. Kuehn, H. S. *et al.* Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science* **345**, 1623–1627 (2014).
38. Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of CD4$^+$ CD25$^+$ regulatory T cells. *Nat. Immunol.* **4**, 330–336 (2003).
39. Lo, B. *et al.* Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. *Science* **349**, 436–440 (2015).
40. Lopez-Herrera, G. *et al.* Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. *Am. J. Hum. Genet.* **90**, 986–1001 (2012).
41. Stannard, J. N. & Kahlenberg, J. M. Cutaneous lupus erythematosus: updates on pathogenesis and associations with systemic lupus. *Curr. Opin. Rheumatol.* **28**, 453–459 (2016).
42. Gathmann, B. *et al.* Clinical picture and treatment of 2,212 patients with common variable immunodeficiency. *J. Allergy Clin. Immunol.* **134**, 116–126.e11 (2014).
43. Lucas, C. L. *et al.* Heterozygous splice mutation in *PIK3R1* causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J. Exp. Med.* **211**, 2537–2547 (2014).
44. Elkaim, E. *et al.* Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: a cohort study. *J. Allergy Clin. Immunol.* **138**, 210–218.e9 (2016).
45. Rao, V. *et al.* Effective 'activated PI3K δ syndrome'-targeted therapy with the PI3Kδ inhibitor leniolisib. *Blood* **130**, 2307–2316 (2017).
46. Jackson, C. E. *et al.* Autoimmune lymphoproliferative syndrome with defective Fas: genotype influences penetrance. *Am. J. Hum. Genet.* **64**, 1002–1014 (1999).
47. Zhang, Q. *et al.* Combined immunodeficiency associated with *DOCK8* mutations. *N. Engl. J. Med.* **361**, 2046–2055 (2009).
48. Biggs, C. M., Keles, S. & Chatila, T. A. DOCK8 deficiency: Insights into pathophysiology, clinical features and management. *Clin. Immunol.* **181**, 75–82 (2017).
49. Bacchelli, C. *et al.* Mutations in linker for activation of T cells (LAT) lead to a novel form of severe combined immunodeficiency. *J. Allergy Clin. Immunol.* **139**, 634–642.e5 (2017).
50. Keller, B. *et al.* Early onset combined immunodeficiency and autoimmunity in patients with loss-of-function mutation in LAT. *J. Exp. Med.* **213**, 1185–1199 (2016).
51. Wilks, A. F. Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. *Proc. Natl Acad. Sci. USA* **86**, 1603–1607 (1989).
52. Darnell, J. J., Kerr, M. & Stark, G. R. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264**, 1415–1422 (1994).
53. Guschin, D. *et al.* A major role for the protein tyrosin kinase JAK1 in the JAK/STAT signal transduction pathway in response to interleukin-6. *EMBO J.* **14**, 1421–1429 (1995).
54. Russell, S. M. *et al.* Interaction of IL-2Rβ and gamma c chains with Jak1 and Jak3: Implications for XSCID and XCID. *Science* **266**, 1042–1045 (1994).
55. Villarino, A. V., Kanno, Y. & Shea, J. J. O. Mechanisms and consequences of Jak–STAT signaling in the immune system. *Nat. Immunol.* **18**, 374–384 (2017).
56. Del Bel, K., Ragotte, R., Saferali, A. & Lee, S. JAK1 gain-of-function causes an autosomal dominant immune dysregulatory and hypereosinophilic syndrome. *J. Allergy Clin. Immunol.* **139**, 2016–2020 (2017).
57. Macchi, P. *et al.* Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature* **377**, 65–68 (1995).
58. Baxter, E. J. *et al.* Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* **365**, 1054–1061 (2005).
59. Scott, L. M. *et al. JAK2* exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N. Engl. J. Med.* **356**, 459–468 (2007).
60. Zheng, J. *et al.* Gain-of-function STAT1 mutations impair STAT3 activity in patients with chronic mucocutaneous candidiasis (CMC). *Eur. J. Immunol.* **45**, 2834–2846 (2015).
61. Liu, L. *et al.* Gain-of-function human *STAT1* mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *J. Exp. Med.* **208**, 1635–1648 (2011).
62. Uzel, G. *et al.* Dominant gain-of-function *STAT1* mutations in *FOXP3* wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *J. Allergy Clin. Immunol.* **131**, 1611–1623 (2013).
63. van de Veerdonk, F. L. *et al. STAT1* mutations in autosomal dominant chronic mucocutaneous candidiasis. *N. Engl. J. Med.* **365**, 54–61 (2011).
64. Kong, X.-F. *et al.* A novel form of human STAT1 deficiency impairing early but not late responses to interferons. *Blood* **116**, 5895–5906 (2010).
65. Dupuis, S. *et al.* Impairment of mycobacterial but not viral immunity by a germline human *STAT1* mutation. *Science* **293**, 300–303 (2001).
66. Koskela, H. *et al.* Somatic STAT3 mutations in large granular lymphocytic leukemia. *N. Engl. J. Med.* **366**, 1905–1913 (2012).
67. Milner, J. D. *et al.* Early-onset lymphoproliferation and autoimmunity caused by germline *STAT3* gain-of-function mutations. *Blood* **125**, 591–599 (2015).
68. Buckley, R., Wray, B. & Belmaker, E. Extreme hyperimmunoglobulinemia E and undue susceptibility to infection. *Pediatrics* **49**, 59–70 (1972).
69. Milner, J. D. *et al.* Impaired T_H17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature* **452**, 773–776 (2008).
70. Winthrop, K. L. *et al.* Herpes zoster and tofacitinib therapy in patients with rheumatoid arthritis. *Arthritis Rheumatol.* **66**, 2675–2684 (2014).
71. Baeten, D. *et al.* Secukinumab, an interleukin-17A inhibitor, in ankylosing spondylitis. *N. Engl. J. Med.* **373**, 2534–2548 (2015).
72. Hartmann, G. Nucleic acid immunity. *Adv. Immunol.* **133**, 121–169 (2017).
73. Picard, C. & Belot, A. Does type-I interferon drive systemic autoimmunity? *Autoimmun. Rev.* **16**, 897–902 (2017).
74. Baechler, E. C. *et al.* Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl Acad. Sci. USA* **100**, 2610–2615 (2003).
75. Crow, Y. J. & Casanova, J.-L. STING-associated vasculopathy with onset in infancy — a new interferonopathy. *N. Engl. J. Med.* **371**, 568–571 (2014).
76. Liu, Y. *et al.* Activated STING in a vascular and pulmonary syndrome. *N. Engl. J. Med.* **371**, 507–518 (2014).
77. Briggs, T. A. *et al.* Spondyloenchondrodysplasia due to mutations in *ACP5*: a comprehensive survey. *J. Clin. Immunol.* **36**, 220–234 (2016).
78. Atkinson, J. Complement deficiency. Predisposing factor to autoimmune syndromes. *Am. J. Med.* **85**, 45–47 (1988).
79. Clancy, R. M. *et al.* Ro60-associated single-stranded RNA links inflammation with fetal cardiac fibrosis via ligation of TLRs: a novel pathway to autoimmune-associated heart block. *J. Immunol.* **184**, 2148–2155 (2010).
80. Savarese, E. *et al.* U1 small nuclear ribonucleoprotein immune complexes induce type I interferon in plasmacytoid dendritic cells through TLR7. *Blood* **107**, 3229–3234 (2006).
81. Yung, S. & Chan, T. M. Anti-DNA antibodies in the pathogenesis of lupus nephritis — the emerging mechanisms. *Autoimmun. Rev.* **7**, 317–321 (2008).
82. Werwitzke, S. *et al.* Inhibition of lupus disease by anti-double-stranded DNA antibodies of the IgM isotype in the (NZB × NZW)F1 mouse. *Arthritis Rheum.* **52**, 3629–3638 (2005).
83. Manderson, A. P. Botto, M. & Walport, M. J. The role of complement in the development of systemic lupus erythematosus. *Ann. Rev. Immunol.* **22**, 431–456 (2004).
84. Litzman, J. *et al.* Early manifestation and recognition of C2 complement deficiency in the form of pyogenic infection in infancy. *J. Paediatr. Child Health* **39**, 274–277 (2003).
85. Ram, S., Lewis, L. A. & Rice, P. A. Infections of people with complement deficiencies and patients who have undergone splenectomy. *Clin. Microbiol. Rev.* **23**, 740–780 (2010).

86. Borzy, M., Gewurz, A., Wolff, L., Houghton, D. & Lovrien, E. Inherited C3 deficiency with recurrent infections and glomerulonephritis. *Am. J. Dis. Child* **142**, 79–83 (1988).
87. Figueroa, J. E. & Densen, P. Infectious diseases associated with complement deficiencies. *Clin. Microbiol. Rev.* **4**, 359–395 (1991).
88. Witte, T. *et al.* Defect of a complement receptor 3 epitope in a patient with systemic lupus erythematosus. *J. Clin. Invest.* **92**, 1181–1187 (1993).
89. Hom, G. *et al.* Association of systemic lupus erythematosus with *C8orf13-BLK* and *ITGAM-ITGAX*. *N. Engl. J. Med.* **358**, 900–909 (2008).
90. Nath, S. K. *et al.* A nonsynonymous functional variant in integrin-αM (encoded by *ITGAM*) is associated with systemic lupus erythematosus. *Nat. Genet.* **40**, 152–154 (2008).
91. Takai, T., Ono, M., Hikida, M., Ohmori, H. & Ravetch, J. Augmented humoral and anaphylactic responses in FcγRII-deficient mice. *Nature* **379**, 346–349 (1996).
92. Willcocks, L. C. *et al.* A defunctioning polymorphism in *FCGR2B* is associated with protection against malaria but susceptibility to systemic lupus erythematosus. *Proc. Natl Acad. Sci. USA* **107**, 7881–7885 (2010).
93. Niederer, H. A. *et al.* Copy number, linkage disequilibrium and disease association in the *FCGR* locus. *Hum. Mol. Genet.* **19**, 3282–3294 (2010).
94. Wu, J. *et al.* A novel polymorphism of FcγRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest.* **100**, 1059–1070 (1997).
95. Sondermann, P. The FcγR/IgG interaction as target for the treatment of autoimmune diseases. *J. Clin. Immunol.* **36**, 95–99 (2016).
96. Van Parijs, L., Ibraghimov, A. & Abbas, A. K. The roles of costimulation and Fas in T cell apoptosis and peripheral tolerance. *Immunity* **4**, 321–328 (1996).
97. Enari, M., Hug, H. & Nagata, S. Involvement of an ICE-like protease in Fas-mediated apoptosis. *Nature* **375**, 78–81 (1995).
98. Shah, S., Wu, E., Rao, V. K. & Tarrant, T. K. Autoimmune lymphoproliferative syndrome: an update and review of the literature. *Curr. Allergy Asthma Rep.* **14**, 10–14 (2014).
99. Rieux-Laucat, F., Le Deist, F., Hivroz, C. & Roberts, I. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* **268**, 1347–1350 (1995).
100. Neven, B. *et al.* A survey of 90 patients with autoimmune lymphoproliferative syndrome related to *TNFRSF6* mutation. *Blood* **118**, 4798–4807 (2011).
101. Fisher, G. H. *et al.* Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* **81**, 935–946 (1995).
102. Watanabe-Fukunaga, R., Brannan, C., Copeland, N., Jenkins, N. & Nagata, S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* **356**, 314–317 (1992).
103. Bride, K. L. *et al.* Sirolimus is effective in relapsed/refractory autoimmune cytopenias: results of a prospective multi-institutional trial. *Blood* **127**, 17–28 (2016).
104. Roos, D. Chronic granulomatous disease. *Br. Med. Bull.* **118**, 53–66 (2016).
105. Roos, D. & de Boer, M. Molecular diagnosis of chronic granulomatous disease. *Clin. Exp. Immunol.* **175**, 139–149 (2013).

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The role of semaphorins in immune responses and autoimmune rheumatic diseases

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Abstract | Semaphorins have a well-characterized role in guiding axon repulsion during development; however, the important contribution of these proteins in immunity is becoming increasingly clear. Immunoregulatory semaphorins, termed 'immune semaphorins', have roles in regulating immune cell activation, differentiation, mobility and migration. These proteins are also intimately associated with the pathogenesis of autoimmune diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). This Review discusses the pathogenic functions of immune semaphorins, as well as the potential use of these molecules as diagnostic markers and therapeutic targets for the treatment of autoimmune diseases.

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The semaphorin family comprise a group of structurally similar molecules characterized by the presence of a Sema domain of ~500 amino acids. Semaphorins were originally identified as neural guidance molecules that lead neuronal axons to their appropriate targets[1]. Since their initial characterization, however, the results of myriad studies have demonstrated that semaphorins function in many physiological processes beyond neuronal guidance, including vascular growth[2,3], regulation of tumour microenvironment[4–6], bone homeostasis[7–9], retinal homeostasis[10,11] and regulation of immune responses[12–18]. Semaphorins that have important roles in immune responses are known as 'immune semaphorins' (REFS 19,20).

Members of the semaphorin family are classified into seven categories: classes 1 and 2 are found in invertebrates, whereas classes 3 to 7 are found in vertebrates. Among the vertebrate semaphorins, class 3 members are secreted, and those in classes 4–7 are membrane-attached (FIG. 1). Some membrane-bound semaphorins (for example, semaphorin 4A (SEMA4A), SEMA4D, SEMA5A, and SEMA7A) can exist as soluble proteins following proteolytic cleavage, whereas others are strictly membrane-bound molecules (for example, SEMA4B). Neuropilins and plexins are the predominant semaphorin receptors[21–23], although several additional kinds of proteins (such as T cell immunoglobulin and mucin domain-containing protein 2 (TIMD-2, also known as TIM-2) and CD72) also participate in semaphorin signalling. Both secreted and membrane-bound semaphorins have roles in multiple aspects of immune responses.

Basic research has shown that semaphorins contribute to the pathogenesis of various autoimmune diseases. For example, SEMA3A, SEMA4A, SEMA4D and SEMA7A have all been implicated in the pathogenesis of multiple sclerosis, a demyelinating disease of the central nervous system[20]. Over the past decade, a number of studies have implicated semaphorins in rheumatic diseases, such as rheumatoid arthritis (RA)[24–31], systemic lupus erythematosus (SLE)[32–37], systemic sclerosis (SSc)[38–40] and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)[41,42]. In addition, several studies have clearly demonstrated the potential of semaphorins as diagnostic and therapeutic targets in these rheumatic diseases[30,31]. In this Review, we summarize the rapidly increasing knowledge of the roles of immune semaphorins, and discuss their pathologic roles and therapeutic implications in autoimmune rheumatic diseases.

Immune functions of semaphorins

Immune semaphorins are expressed in a wide range of immune cells and have roles in various immune responses (FIG. 2), as discussed in this section.

Class 3 semaphorins

Class 3 semaphorins, which are secreted semaphorins, typically function through binding class A plexins (plexins A1–A4) and, unlike classes 4–7 semaphorins, require neuropilins as obligate co-receptors for this interaction. Additionally, SEMA3E binds to plexin D1 in a neuropilin-1 (NRP1)-independent manner. Of this

Key points

- Semaphorins have important roles in regulating various responses of the immune system.
- Semaphorins are associated with the pathogenesis of autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).
- Semaphorins show promise as diagnostic markers and/or therapeutic targets for the treatment of rheumatic diseases.

family of semaphorins, SEMA3A and SEMA3E function as immune semaphorins (as discussed in this section); for example, SEMA3A promotes the migration of dendritic cells (DCs) from the periphery to draining lymph nodes[18] and SEMA3E regulates the trafficking of thymocytes during differentiation. Cumulative findings indicate that class 3 semaphorins are also involved in the pathogenesis of autoimmune diseases (discussed in a later section). For instance, SEMA3A functions as a negative regulator of lymphocytic function in the pathogenesis of RA[25] (T cells), SLE[34] (B cells), and SSc[40] (regulatory T (T_{reg}) cells); SEMA3C is a nerve-repellant factor in the RA synovium[24], and SEMA3E serves as an angiogenic factor for microvasculature in the pathogenesis of SSc[43].

SEMA3A and T cell proliferation. SEMA3A is synthesized by activated DCs and T cells, as well as by multiple types of cancer cells[44]. SEMA3A downregulates T cell proliferation via the NRP1–plexin A4 receptor complex by blocking mitogen-activated protein kinase signalling and interfering with the cell-cycle progression of T cells[44]. T cells harbouring a mutant form of NRP1 that lacks the binding site for SEMA3A and *Plxna4*-deficient T cells proliferate more rapidly *in vitro* following T cell receptor (TCR) stimulation than wild-type controls[45]. In addition, *Plxna4*$^{-/-}$ mice exhibit more active T cell-mediated immune responses than wild-type mice, for example, in models such as experimental autoimmune encephalomyelitis (EAE)[45].

SEMA3A and macrophage activation. Although SEMA3A has a suppressive effect on T cells, this protein acts as a positive regulator in the innate immune system. *Plxna4*$^{-/-}$ macrophages produce reduced levels of inflammatory cytokines following exposure to Toll-like receptor (TLR) agonists or bacteria[46]. This phenomenon can be attributed to a reduction in TLR-induced RAS-related C3 botulinum toxin substrate (RAC1) activation, which in turn diminishes activation of c-Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB)[46]. The mechanism by which plexin A4 targets this pathway is unclear but, consistent with this *in vitro* observation, *Plxna4*$^{-/-}$ mice are less prone than their wild-type counterparts to develop polymicrobial peritonitis following cecal ligation and puncture[46]. Administration of exogenous SEMA3A considerably increases cytokine production in response to TLR agonists or bacterial sepsis in mice, supporting the idea that SEMA3A activates innate immune cells. Furthermore, anti-SEMA3A antibody administration improves the survival rate of mice with lipopolysaccharide-induced sepsis[47]. Taken together, these findings demonstrate that SEMA3A has various types of effects on immune responses: negative in the case of T cell mediated specific immunity, but positive in the case of innate immunity.

SEMA3A and dendritic cell transmigration. SEMA3A is also involved in DC transmigration across the lymphatics. This activity is mediated via its receptor plexin A1, which is expressed on the rear sides of DCs during DC migration[18]. Lymphatic endothelial cells secrete SEMA3A, which then interacts with NRP1 and plexin A1 on the surface of DCs; this association induces phosphorylation of myosin light chain, causing the cell body of the DC to constrict and enabling the DCs to migrate through small gaps between endothelia. Consistent with these findings, T cell priming, a DC-dependent process that occurs in the lymph nodes, is impaired in either *Sema3a*$^{-/-}$ or *Plxna1*$^{-/-}$mice, and findings from *in vivo* adoptive transfer models indicate that SEMA3A secreted by lymphatic endothelial cells promotes DC trafficking from the periphery to draining lymph nodes[18].

SEMA3E, thymocyte development and dendritic cell recruitment. SEMA3E, which is mainly expressed in the thymus medulla, has an important role in thymocyte development[48]. SEMA3E binds to plexin D1 expressed by double-positive ($CD4^+CD8^+$) thymocytes and inhibits CC-chemokine receptor 9 (CCR9)-mediated thymocyte chemotaxis towards the thymus cortex. Plexin D1 levels on the surface of thymocytes decrease over the course of thymocyte development, from the double-positive to single-positive stages. This SEMA3E–plexin D1 axis contributes to the well-directed migration of maturing thymocytes and the orderly formation of thymic corticomedullary structure. Indeed, transplantation studies fetal liver cells from *Plxnd1*$^{-/-}$ embryos show that $CD69^+$ double-positive thymocytes are abundantly localized in the cortex, and that the boundary between double-positive and single-positive thymocytes at the corticomedullary junction is disrupted[48]. However, whether abnormal thymocyte development due to a lack of SEMA3E–plexin D1 signalling could lead to immune pathology remains to be elucidated.

SEMA3E also has a regulatory role in recruiting $CD11b^+$ DC subsets, which are functionally activated DCs that promote T helper 2 (T_H2) and T helper 17 (T_H17) responses, to the lung. *Sema3e*$^{-/-}$ mice show increased numbers of pulmonary $CD11b^+$ DCs in an experimental model of house dust mite (HDM)-induced allergic asthma[49]. In addition, recombinant SEMA3E proteins ameliorate the pathological features of allergic airway disease[50].

Class 4 semaphorins

Class 4 semaphorins are all membrane-bound, but SEMA4A and SEMA4D can be cleaved from the cell surface to yield soluble forms. Class 4 semaphorins can directly bind to class B plexins to mediate their effects[51,52];

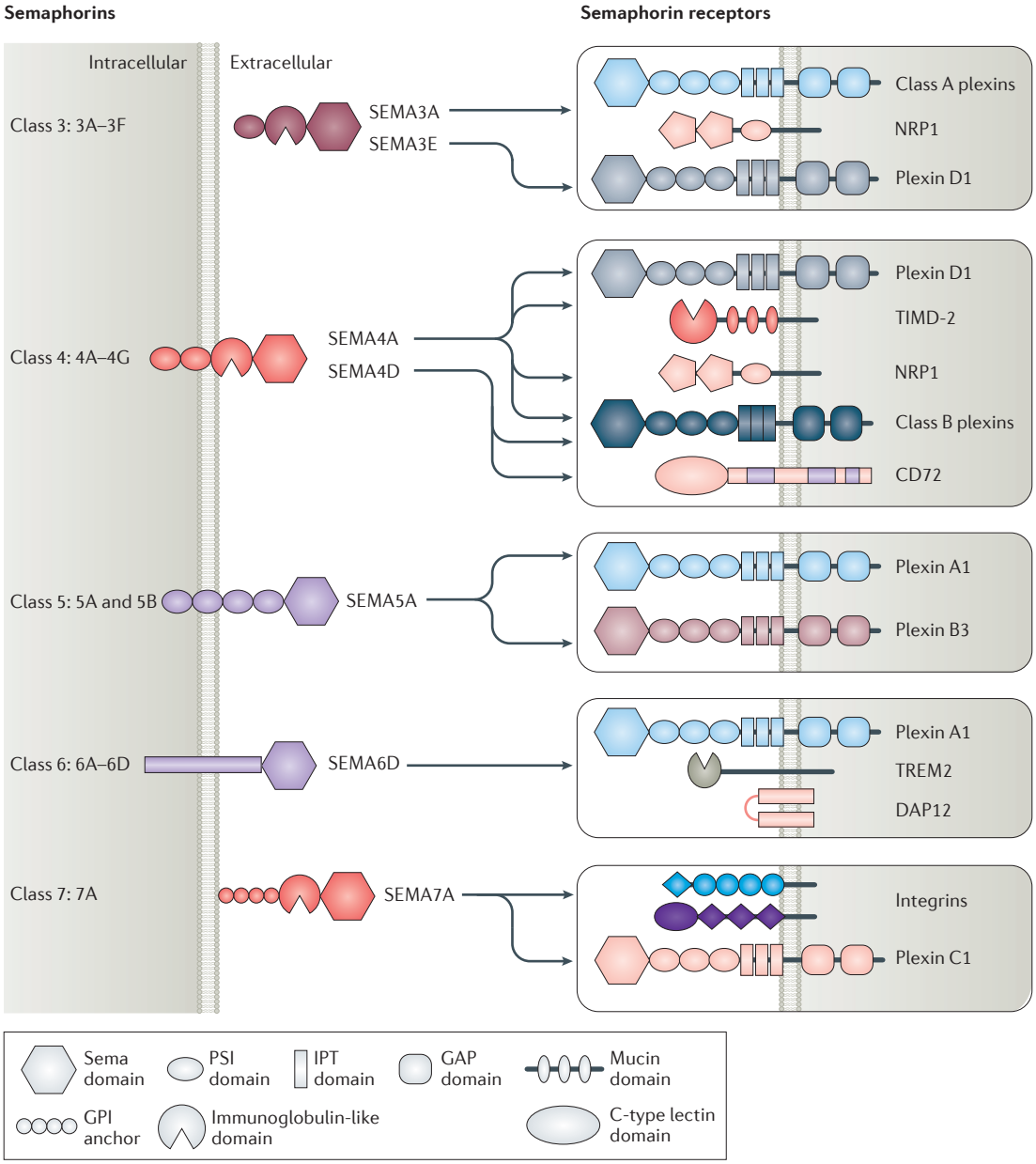

Figure 1 | **Immune semaphorin and receptor interactions.** SEMA3A interacts with complexes of neuropilin 1 (NRP1) and class A plexins. SEMA3E binds to plexin D1 in a NRP1-independent manner. SEMA4A interacts directly with class B plexins, plexin D1, T cell immunoglobulin and mucin domain-containing protein 2 (TIMD-2, also known as TIM-2) and NRP1. The receptor for SEMA4B is currently unknown. SEMA4D binds to class B plexins and CD72 in the immune system. The receptors for SEMA5A are plexin A1 and plexin B3. SEMA6D exerts various biological activities through plexin A1, depending on its co-receptor; in the immune system, SEMA6D binds to the plexin A1–triggering receptor expressed on myeloid cell 2 (TREM2)–DNAX-activating protein 12 (DAP12, also known as TYRO protein tyrosine kinase-binding protein) receptor complex to transduces signals. SEMA7A associates with integrins and plexin C1 in the immune system. GAP domain, GTPase-activating domain; GPI, glycophosphatidylinositol; IPT domain, Ig-like, plexins, transcription factors domain; PSI domain, plexin–semaphorin–integrin domain. Adapted from *Nat. Rev. Immunol.* **13**, 802–814 (2013).

additionally, SEMA4A can bind to NRP1 (REF. 53) and plexin D1 (REFS 54,55), and SEMA4A and SEMA4D can interact with TIMD-2 (REF. 15) and CD72 (REF. 13), respectively. In addition, membrane-bound SEMA4D functions as a direct signalling receptor for plexin B1 and B2 ligands on some immune cells (for example, B cells, γδT cells and neutrophils, as discussed in this section). Both the secreted and membrane-bound class 4 semaphorins have critical roles in regulating lymphocyte activation and immune homeostasis[14,15] (discussed in this section). Both the secreted and membrane-bound forms of class 4 semaphorins also have roles in autoimmunity (as discussed in a later section). For instance, soluble SEMA4D enhances the production of inflammatory cytokines by

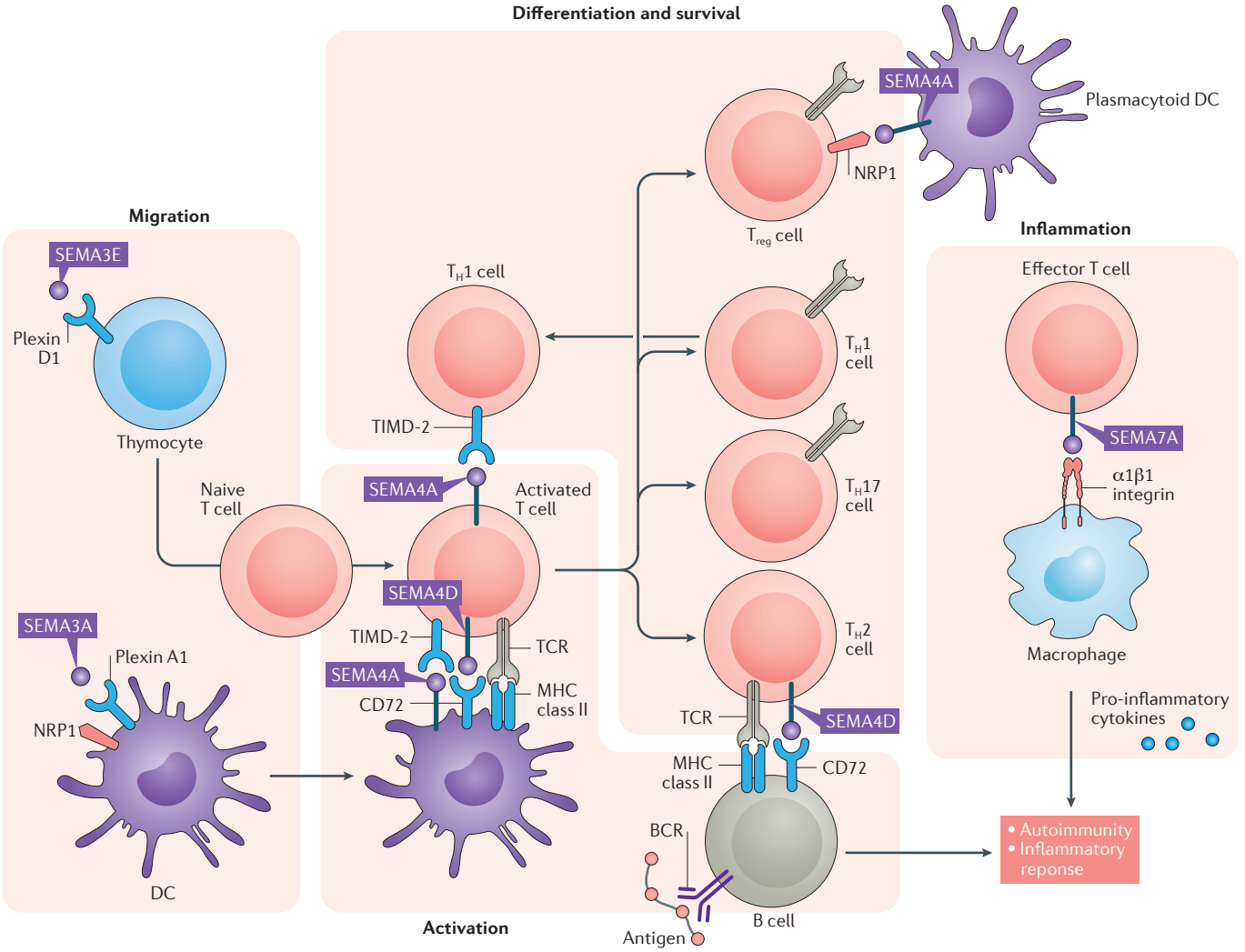

Figure 2 | **The involvement of immune semaphorins in various aspects of immune responses.** During dendritic cell (DC) transmigration, SEMA3A binds to the plexin A1–neuropilin 1 (NRP1) receptor complex expressed on the rear sides of DCs, inducing their transmigration into the lymphatics. SEMA3E binds to plexin D1 expressed on thymocytes and contributes to thymocyte development by regulating thymocyte migration. SEMA4A regulates the differentiation of $CD4^+$ T cells by amplifying T helper 1 (T_H1) cell differentiation through the binding of T cell immunoglobulin and mucin domain-containing protein 2 (TIMD-2, also known as TIM-2) on T_H1 cells. SEMA4A promotes the survival of regulatory T (T_{reg}) cells by binding to NRP1 expressed by these cells. In the initial phase of T cell immune responses, SEMA4A expressed by DCs promotes the activation of T cells capable of recognizing alloantigens presented on DCs, through binding to TIMD-2 on activated T cells. SEMA4D expressed by T cells positively regulates humoral immune responses by activating B cells via CD72. SEMA4D expressed on T cells interacts with CD72 on DCs and promotes DC activation and maturation. SEMA7A is expressed on activated T cells and stimulates macrophages via integrins to produce pro-inflammatory cytokines. BCR, B cell receptor; TCR, T cell receptor.

monocytes[30] (RA) and induces antibody production by B cells[32] (SLE). Membrane-bound SEMA4D prevents excessive activation of neutrophils as a functional receptor[41] (AAV).

SEMA4A and differentiation of T helper cells. SEMA4A is expressed at high levels in T helper 1 (T_H1) cells, and has an important role in T_H1 cell differentiation[15,16]. SEMA4A-Fc protein binds to TIMD-2 expressed on activated T cells ($CD62L^{hi}CD4^+$ naive T cells stimulated with anti-CD3 antibody and anti-CD28 antibody) and amplifies T_H1 differentiation[15]. In addition, the levels of SEMA4A expression on T cells are enhanced throughout T_H1 differentiation, but not T_H2 differentiation. *In vitro* differentiation of T cells from *Sema4a*$^{-/-}$ mice into T_H1 cells is severely impaired[16], suggesting that increased expression of SEMA4A in T_H1-differentiating cells further promotes T_H1 differentiation. Under these circumstances, SEMA4A might function in an autocrine manner or through cognate T cell–T cell contacts. Consistent with this finding, mice lacking *Sema4a* exhibit diminished T_H1 responses and enhanced T_H2 responses[16]. These animals

are also less prone to develop EAE, a condition mediated by T_H1 cell and T_H17 cell responses to myelin oligodendrocyte glycoprotein (MOG) peptides, and anti-Sema4A antibody can block the development of EAE[15]. Both *Sema4a*$^{-/-}$ mice and *Timd2*$^{-/-}$ mice have a dysregulated T_H2 cytokine response and exacerbated lung inflammation in an experimental mouse model of asthma[56]. In a different study using the same model of experimental asthma, recombinant SEMA4A treatment suppressed asthmatic inflammation, but anti-TIMD-2 antibody administration only partially increased disease severity[57]. These data raise the possibility that SEMA4A interacts with binding partners other than TIMD-2 in this asthma model.

SEMA4A and regulatory T cell stability. SEMA4A interacts with NRP1 on the surface of T_{reg} cells, activating these cells and promoting their survival in inflamed or cancerous tissues[53]. Ligand-bound NRP1 recruits phosphatase and tensin homologue (PTEN), limiting activation of the serine-threonine kinase AKT, both in the cell body and at immunologic synapses, and facilitating nuclear translocation of forkhead box O3A (FOXO3A), a transcription factor critical for T_{reg} cell development. Mice with a T_{reg} cell-restricted deletion of NRP1 do not develop autoimmune disorders, suggesting that NRP1 is dispensable for the suppression of autoimmunity and maintenance of immune homeostasis. Thus, the SEMA4A–NRP1 axis is crucially involved in the maintenance of T_{reg} cell stability, and has therapeutic potential for limiting tumour-induced tolerance mediated by T_{reg} cells without affecting autoimmunity.

SEMA4A and dendritic cell–T cell interactions. SEMA4A was originally cloned from a complementary DNA (cDNA) library of DCs[15]. SEMA4A constitutively expressed by DCs is critical for T cell–mediated immune responses, possibly by interacting with TIMD-2. SEMA4A-deficient DCs have an impaired ability to stimulate allogeneic T cells compared with wild-type DCs. By contrast, $CD4^+$ T cells from *Sema4a*$^{-/-}$ mice and wild-type mice show comparable levels of proliferation when cultured with allogeneic DCs in mixed lymphocyte reactions[16]. These data suggest that SEMA4A expressed by DCs promotes the activation of T cells.

SEMA4B and basophil–T cell interactions. SEMA4B is expressed by both T cells and B cells and negatively regulates the function of basophils through T cell–basophil interactions[58]. Basophils produce IL-4 in response to helminthic infections, and mediate B cell memory responses and T_H2 skewing of the T cell population. SEMA4B decreases IL-4 secretion by basophils, and T cell-derived SEMA4B inhibits basophil-mediated T_H2 cell skewing. Although *Sema4b*$^{-/-}$ mice exhibit no functional lymphocyte or DC abnormalities, these mice have increases levels of serum IgE compared with their wild-type counterparts owing to increased basophil-mediated B cell memory responses. The receptor for SEMA4B remains unknown, but these findings indicate that SEMA4B negatively regulates basophil-mediated T_H2 and humoral memory responses[58].

SEMA4D and B cell proliferation. SEMA4D (also known as CD100) was the first semaphorin to be characterized as an immune semaphorin[12]. SEMA4D is expressed at low basal levels in mouse B cells, but upon exposure of these cells to anti-CD40 antibodies or lipopolysaccharide, Sema4D expression is upregulated[13]. In cultured mouse[13] and human[59] B cells, elevated expression of SEMA4D promotes B cell proliferation and antibody generation via its receptor CD72. Similarly, B cells from mice lacking SEMA4D exhibit impaired proliferation and dysregulated antibody production[60]. In its cytoplasmic domain, CD72 contains two immunoreceptor tyrosine–based inhibitory motifs (ITIMs). The SEMA4D–CD72 axis turns off the inhibitory signals from ITIM in CD72, and maintains appropriate B cell receptor (BCR) signalling[61], which promotes growth of the B cell population; accordingly, SEMA4D is highly expressed by germinal center B cells, which are maturated and secrete high-affinity antibodies[62]. Plexin B1 expressed by human bone marrow stromal cells, follicular DCs and activated T cells promotes B cell proliferation and lifespan[63] by binding to SEMA4D on B cells. This suggests that reverse signalling from plexin B1 to SEMA4D is also associated with the maintenance of B cell responses. Thus, these data indicate that SEMA4D is involved in regulating B cell function, as well as appropriate 'tuning' of BCR signals.

SEMA4D and T cell–DC cross-interactions. T cells express large quantities of SEMA4D, whereas DCs express its receptor CD72 (REFS 13,64). In addition to having reduced antibody levels, mice lacking *Sema4d* are less prone to developing EAE than their wild-type counterparts owing to an impairment in their ability to generate MOG-specific T cells[64]. However, the T cells of these mice exhibit normal responses upon stimulation with anti-CD3 antibody. Furthermore, recombinant soluble SEMA4D does not directly affect T cell activation *in vitro*[14] but instead upregulates the expression of CD80, CD86 and major histocompatibility complex (MHC) class II on the surface of DCs. Recombinant SEMA4D also increases the immunogenicity of DCs induced by CD40 stimulation[64]. These data suggest that SEMA4D expressed on T cells interacts with CD72 on DCs and promotes DC activation and maturation, which in turn augments T cell activation.

SEMA4D and γδ T cell immunity. SEMA4D functions as a receptor for plexin B2 ligand on activated γδ T cells. The SEMA4D-plexin B2 axis is involved in cytoskeleton remodelling in γδ T cells by modulating α6β4 integrin expression. Round-shaped γδ T cells can secrete cytokines and growth factors and promote skin wound healing[52]. *Sema4d*$^{-/-}$ mice have defective dermatotic γδ T cell responses to keratinocyte damage, resulting in delayed healing of cutaneous wounds[52].

SEMA4D, mast cell function and neutrophil activation. On human mast cells, SEMA4D associates with CD72 and inhibits KIT-mediated proliferation and the expression of CCL2 (REF. 65). The SEMA4D–CD72 axis might also have an important role in negatively regulating KIT-mediated mast cell responses.

SEMA4D is strongly expressed in human neutrophils. Similar to its function in γδ T cells, neutrophil-surface SEMA4D functions as a receptor for plexin B2 on endothelial cells, and this interaction negatively regulates the inflammatory activation of neutrophils[41]. Furthermore, SEMA4D is proteolytically cleaved and released from the cell surface upon neutrophil activation. Reduction in the level of SEMA4D might amplify inappropriate neutrophil-mediated inflammatory responses.

Class 5 semaphorins

The class 5 semaphorin SEMA5A is expressed by oligodendrocytes and inhibits axon growth in the nervous system[66]. In addition, SEMA5A promotes angiogenesis by increasing endothelial cell proliferation and decreasing apoptosis[67]. Receptors for SEMA5A are plexin A1 (REF. 68) and plexin B3 (REF. 69). SEMA5A is also involved in the pathogenesis of RA (discussed in a later section), as a promoting factor for the proliferation of inflammatory T cells and NK cells[27].

Class 6 semaphorins

Class 6 semaphorins are membrane-bound semaphorins with long cytoplasmic tails; these semaphorins directly bind to class A plexins. Among the class 6 semaphorins, SEMA6D can function as an immune semaphorin; T cells, B cells and natural killer (NK) cells all express SEMA6D, whereas DCs specifically express plexin A1, which acts as a receptor for SEMA6D (REF. 8). To date, no studies have implicated SEMA6D in autoimmune rheumatic disease pathogenesis but, as discussed in this section, this semaphorin has been implicated in other autoimmune diseases (such as EAE).

$CD4^+$ T cells express SEMA6D following TCR-stimulation *in vitro*[70]. Blocking SEMA6D–ligand interactions with either an anti-SEMA6D monoclonal antibody or a SEMA6D–Ig fusion protein diminishes late-phase activation of these cells by decreasing phosphorylation of the linker of activated T cells (LAT) protein and CRK-like protein (CRKL), a substrate of Abl kinase[70]. Thus, the activity of SEMA6D contributes to the endogenous signalling in $CD4^+$ T cells. However, given that mice lacking *Sema6d* exhibit no dysfunction in T cell priming[18], the involvement of SEMA6D in the course of *in vivo* physiological T cell responses is unclear.

The recombinant SEMA6D protein binds to DCs, activating them through plexin A1, and ultimately upregulating the production of IL-12. Mice lacking *Plxna1* have defects in the production of antigen-specific T cells, and consequently are less prone to develop EAE.

Class 7 semaphorins

Of the class 7 semaphorins, SEMA7A has immune functions; in addition to binding to plexin C1, this semaphorin can also binds to integrins in the nervous and immune systems[17,71]. As well as a role in immune responses (discussed in this section), SEMA7A has also been implicated in autoimmunity; for example, this semaphorin promotes the differentiation of T_H1 and T_H17 cell responses and the proliferation of lung fibrocytes in the pathogenesis of RA[31] and SSc-related lung fibrosis[38], respectively (discussed in a later section).

SEMA7A, also known as CD108, is a glucose-6-phosphate isomerase (GPI)-anchored protein containing an integrin-binding motif in its Sema domain. SEMA7A is expressed in activated T cells, accumulates at the immunological synapse between T cells and macrophages, and stimulates macrophages by interacting with α1β1 integrin[17]. The clustering of α1β1 in macrophages at the immunological synapse enables firm adhesion between T cells and macrophages. Furthermore, SEMA7A–integrin binding promotes the generation of pro-inflammatory cytokines, such as IL-6 and TNF. Accordingly, mice lacking *Sema7a* are less likely to develop conditions associated with T cell-mediated immune responses, including hapten-induced contact hypersensitivity and EAE.

Semaphorins in autoimmune diseases

Given the important functions of immune semaphorins in immune responses, these molecules are now considered to have important roles in autoimmune diseases. In the past decade, multiple studies using clinical samples and preclinical disease models have yielded an increasing amount of data regarding the diagnostic and therapeutic potential of semaphorins.

Rheumatoid arthritis

RA is an autoimmune disease characterized by synovial inflammation. In RA, disease progression leads to the destruction of joint tissues, including articular cartilage and bone. Both genetic and environmental factors contribute to the development of autoimmunity, which is associated with the presence of pathogenic autoantigens (for example, citrullinated proteins) in the serum and tissues. Although the pathogenesis of persistent synovitis remains incompletely understood, several studies have implicated semaphorins in RA pathogenesis (TABLE 1).

Class 3 semaphorins. The first study to demonstrate a relationship between semaphorins and RA pathogenesis was reported by Miller *et al.*[24]. In this study, immunohistochemistry analysis revealed that SEMA3C expression was increased in macrophages and fibroblasts derived from the synovial tissue of patients with RA in comparison with healthy individuals and patients with osteoarthritis (OA)[24]. These patients with RA had lower densities of sympathetic nerve fibres and higher densities of $SEMA3C^+$ cells in their synovium than did patients with OA. This observation suggests that SEMA3C secreted by synovial macrophages and fibroblasts is a chemorepellent factor and directs sympathetic nerve fibres out of synovial tissue, which might be one of the mechanisms underlying the chronic symptoms of RA (FIG. 3a). Further *in vivo* studies are needed to elucidate the pathological and therapeutic potential of SEMA3C in RA.

The expression of SEMA3A is lower in $CD4^+$ T cells derived from patients with RA than in those derived from healthy donors, whereas the expression of NRP1 (the SEMA3A receptor) is higher[25]. Activation of the

Table 1 | **Involvement of immune semaphorins-associated molecules in the pathogenesis of rheumatoid arthritis**

Ligands	Ligand-expressing cells	Binding partners	Binding partner-expressing cells	Functions	Diagnostic relevance	Therapeutic relevance	Refs
SEMA3A	$CD4^+$ T cells	NRP1	$CD4^+$ T cells	Suppresses T cell response	Low SEMA3A and high Nrp1 levels in $CD4^+$ T cells from patients with RA	SEMA3A protein	25
VEGF	Not defined	NRP1	Synovial cells	Regulates the growth and proliferation of synovial cells	Not shown	Anti-NRP1 peptide	72
SEMA3C	Synovial macrophages and fibroblasts	Not defined	Not defined	Acts as a chemorepellant factor to direct sympathetic nerve fibres out of the synovial tissue	Not shown	Not shown	24
SEMA4A	Synovial tissue	Plexin B1	Synovial fibroblasts	• Promotes the invasion of RA FLSs into the synovium • Promotes IL-6 production by FLS	High SEMA4A levels in the synovial tissue and fluid of patients with RA	Not shown	28,29
SEMA4D	Synovial lymphocytes	CD72	Monocytes	Induces pro-inflammatory cytokine production by monocytes	High SEMA4D levels in the synovial fluid and serum of patients with RA	SEMA4D-blocking antibody	30
SEMA5A	PBMCs?	Plexin A1 and Plexin B3	T cells and/or NK cells	Promotes T cell and NK cell proliferation	High SEMA5A serum levels of patients with RA	Not shown	27
SEMA7A	$CD4^+$ T cells and $CD14^+$ monocytes	• Not defined for $CD4^+$ T cells • β1-integrin ($CD14^+$ monocytes)	$CD4^+$ T cells and $CD14^+$ monocytes	• Promotes T_H1 and T_H17 cell differentiation • Induces pro-inflammatory cytokine production by monocytes	High SEMA7A levels in the synovial fluid and serum of patients with RA	SEMA7A-blocking antibody	31

FLS, fibroblast-like synoviocyte; NK cell, natural kill cell; NRP1, neuropilin 1; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SEMA, semaphorin; T_H1, T helper 1; T_H17; T helper 17; VEGF, vascular endothelial growth factor.

SEMA3A–NRP1 axis induces IL-10 secretion by $CD4^+$ T cells, promoting the suppressive activity of $CD4^+$ T cells (FIG. 3b); this activity can be inhibited with SEMA3A-blocking antibodies. In a mouse model of RA, treatment with a SEMA3A-encoding plasmid almost completely alleviated joint inflammation and decreased levels of pro-inflammatory cytokines such as IFNγ and IL-17[25]. Thus, the SEMA3A–NRP1 axis has a key role in regulating T cell–mediated RA inflammation.

NRP1 is also as a co-receptor for vascular endothelial growth factor (VEGF), an important mediator of angiogenesis and cell survival. Treatment with an anti-NRP1 peptide directly blocked the binding of VEGF to NRP1 on fibroblast-like synoviocytes (FLS) isolated from the synovial tissue of patients with RA, thereby diminishing the VEGF-induced growth and proliferation of these cells and also decreasing cell survival; the anti-NRP1 peptide also prevented arthritis in a collagen-induced arthritis (CIA) mouse model[72]. This study suggests that anti-NRP1 peptide could be a potential therapy for RA. However, NRP1 also enhances the longevity and suppressive functions of T_{reg} cells in mice[53]. Therefore, the blockade of NRP1 proteins might also interfere with the suppressive functions of T_{reg} cell. Further careful evaluation is needed to clarify the effectiveness of NRP1 blockade for the treatment of autoimmune diseases.

Class 4 semaphorins. SEMA4D has also been implicated in the pathogenesis of RA[30]. This semaphorin is thought to exacerbate the inflammatory responses of patients with RA via a positive-feedback loop involving soluble SEMA4D, pro-inflammatory cytokines (IL-6 and TNF), and a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) (FIG. 3c). Soluble SEMA4D levels are elevated in the serum and synovial fluid of patients with RA, compared with those from patients with OA[30]. The increased levels of soluble SEMA4D are produced by an ADAMTS4-mediated proteolytic mechanism, and the resultant soluble SEMA4D in turn induces the production of IL-6 and TNF by monocytes, suggesting the existence of an inflammatory activation loop in RA pathogenesis. Treatment with an anti-SEMA4D antibody prevented the development of arthritis in a CIA mouse model. Furthermore, serum TNF and IL-6 levels were significantly reduced in anti-SEMA4D antibody–treated mice[30]. These results indicate that blocking SEMA4D might be a useful strategy for RA management. An anti-SEMA4D antibody (VX15/2503) has already shown promise in phase I clinical trials of patients with advanced solid tumour (NCT01313065)[73] and multiple sclerosis (NCT01764737)[74]. This antibody was well tolerated in both studies, and is expected to prevent tumour angiogenesis and the recruitment of

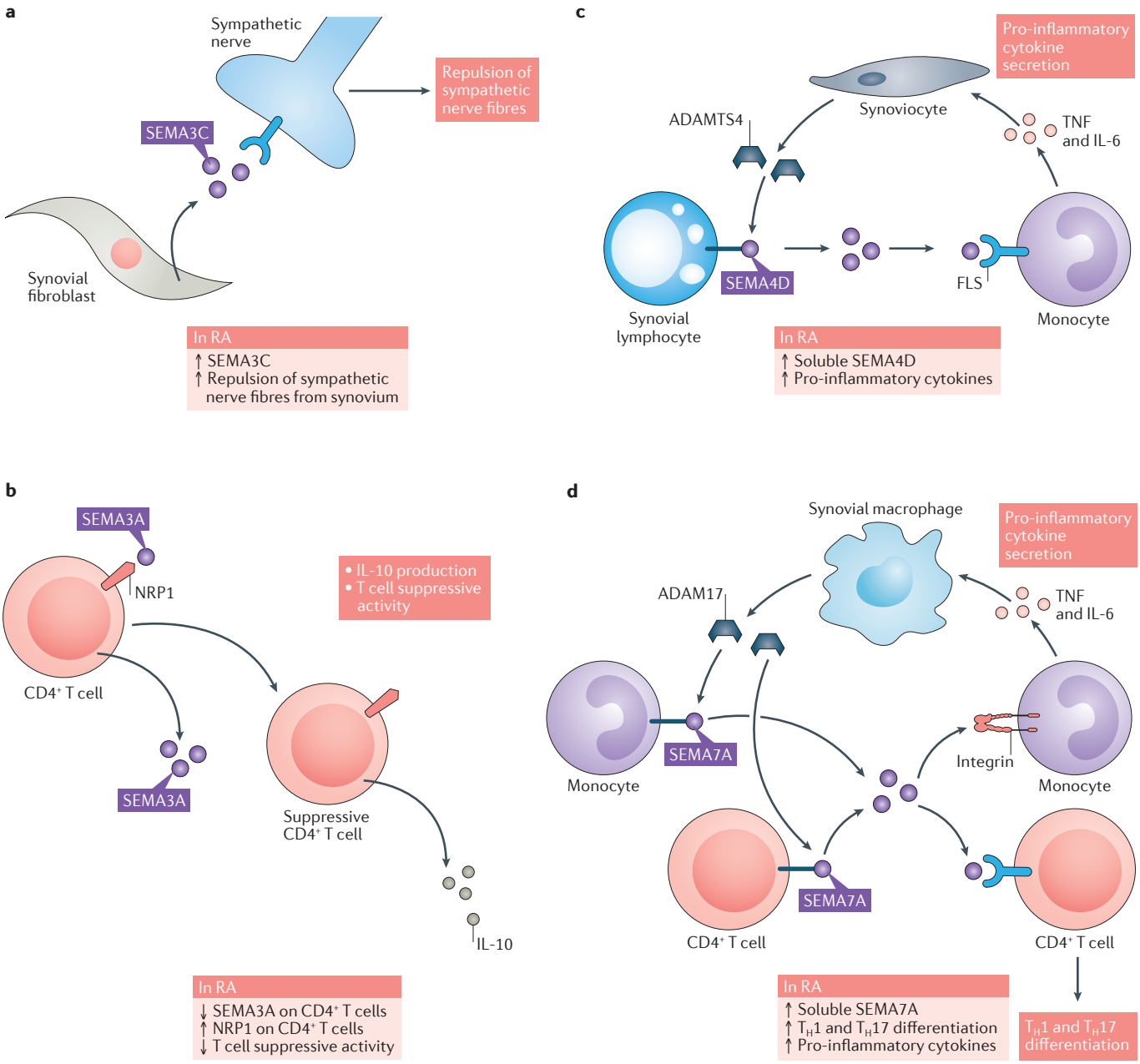

Figure 3 | **The involvement of immune semaphorins in rheumatoid arthritis pathogenesis. a** | SEMA3C is secreted by synovial macrophages and fibroblast-like synopviocytes (FLSs) in patients with rheumatoid arthritis (RA), and repels sympathetic nerve fibres from the synovial tissue. **b** | The SEMA3A–neuropilin 1 (NRP1) axis induces the suppressive activity of $CD4^+$ T cells; expression of SEMA3A is very low in $CD4^+$ T cells derived from patients with RA, resulting in defects in the suppressive T cell response to inflammation. **c** | In the synovium of patients with RA, SEMA4D is proteolytically cleaved from the cell surface of synovial lymphocytes by a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4), yielding the soluble form of SEMA4D. Soluble SEMA4D induces the production of inflammatory cytokines by monocytes. **d** | In the synovium of patients with RA, SEMA7A is proteolytically cleaved from the cell surface of $CD4^+$ T cells and monocytes by disintegrin and metalloproteinase 17 (ADAM17). Soluble SEMA7A is involved in T helper 1 (T_H1) and T helper 17 (T_H17) cell differentiation, and promotes the generation of pro-inflammatory cytokines by monocytes.

tumour associated macrophages (TAMs) in antitumour immunity[75,76], and also impair inflammatory responses and demyelination in multiple sclerosis[74]. However, the long-term feasibility of using this anti-SEMA4D antibody as a therapeutic agent remains unknown.

Class 5 and class 7 semaphorins. Patients with RA, in particular those positive for rheumatoid factor or anti-cyclic citrullinated peptide (CCP), have elevated serum concentrations of soluble SEMA5A compared with patients with SLE, patients with Sjögren syndrome or

healthy individuals[27]. Soluble SEMA5A strongly induces the proliferation of T cells and NK cells and increases the secretion of T_H1 and T_H17 pro-inflammatory cytokines by both types of cells *in vitro*, indicating that SEMA5A might contribute to the pathogenesis of RA through antigen-independent T cell and NK cell activation[27].

Levels of soluble SEMA7A are elevated in both the serum and synovial fluid of patients with RA compared with those in the patients with OA or healthy individuals, in whom $CD4^+$ T cell and monocyte cell surface SEMA7A are cleaved by disintegrin and metalloproteinase 17 (ADAM17)[31]. Soluble SEMA7A induces T-bet and retinoic acid receptor–related orphan nuclear receptor γt (RORγt) upregulation in $CD4^+$ T cells, which promote differentiation into T_H1 and T_H17 subclasses, respectively. Soluble SEMA7A also induces the production of IL-6 and TNF by monocytes, and IL-6 and TNF in turn stimulates ADAM17 secretion by synovial macrophages, suggesting the existence of an inflammatory activation loop in RA pathogenesis, similar to the positive feedback mechanism involving SEMA4D (FIG. 3d). Treatment with an anti-SEMA7A antibody attenuated arthritis scores and paw swelling in mice with CIA compared with control mice[31], suggesting SEMA7A could be a therapeutic target in RA.

Together, these findings indicate that classes 4–7 membrane-bound semaphorins have a tendency to be cleaved in patients with RA, especially during local inflammatory responses in the synovium. Such soluble forms of semaphorins function as pro-inflammatory molecules that evoke inappropriate immune reactions, such as the activation of T_H1 cell and T_H17 cell responses, suggesting that soluble forms of semaphorins could be used as surrogate markers for RA activity, and that neutralizing soluble semaphorins or blocking semaphorin receptors could be potential therapeutic options for RA.

Systemic lupus erythematosus

Research during the past decade has demonstrated the involvement of immune semaphorins in autoimmune rheumatic diseases other than RA (TABLE 2). SLE is a prototypic systemic autoimmune disease that affects multiple organs. The symptoms and severity of SLE vary between individuals and the cause of the disease is not fully understood.

CD72, a receptor for SEMA4D, has been implicated in SLE pathogenesis[32]. The expression of CD72 is lower in B cells from patients with lupus nephritis than in those from healthy individuals, whereas SEMA4D expression in T cells is unaltered[32]. CD72 expression in B cells is associated with the differentiation of these cells. Furthermore, IgG class switching is evident in B cells from patients with lupus nephritis compared with cells from healthy individuals, especially in B cells from patients who have progressed to the later and more severe stages of disease. Thus, decreased expression of CD72 on B cells is considered to reflect the increase of class switching of B cells, as well as being associated with B cell differentiation, suggesting that CD72 is a useful disease marker associated with class switching of B cells in lupus nephritis.

NRP1 (REF. 33), SEMA3A (REF. 34) and soluble CD72 (REF. 36) have also been implicated in SLE pathogenesis. Immunohistological analysis suggests that the expression levels of NRP1 are higher in the glomeruli of patients with lupus nephritis than in those from healthy individuals. In these patients, NRP1 deposits are localized to damaged glomerular areas, and positively correlate with clinical and pathological parameters of renal disease such as serum creatinine, proteinuria and disease activity index scores. Thus, NRP1 is a potential marker for differentiating focal versus diffuse lupus nephritis. Serum levels of SEMA3A are lower in patients with SLE than in patients with RA and also in comparison with serum levels in healthy individuals[34]. SLE disease severity, as measured by the extent of renal damage and the level of serum anti-cardiolipin antibodies, negatively correlates with serum concentrations of SEMA3A. In culture, the presence of Sema3A decreases the expression of TLR9 in CpG-oligodeoxynucleotide (CpG-ODN)-stimulated B cells derived from patients with SLE[34]. These results suggest that SEMA3A might have a regulatory role in SLE pathogenesis through the inhibition of TLR9-mediated B cell responses. The soluble form of CD72 is present at higher levels in patients with SLE than in both patients with RA and healthy individuals[36]. Furthermore, soluble CD72 levels are higher in patients with lupus nephritis than in patients without renal involvement, and soluble CD72 levels are also higher in patients with autoantibodies such as anti-double-stranded DNA (anti-dsDNA) antibodies or anti-cardiolipin antibodies. Accordingly, elevated soluble CD72 levels could be a potential biomarker for renal involvement in SLE.

Thus, CD72 seems to be an important molecule involved in SLE pathogenesis. As mentioned above, basic research on the SEMA4D–CD72 axis has clearly demonstrated that CD72 has an important role in fine-tuning BCR signalling. Alterations in CD72 expression might be involved in the dysregulated B cell responses that contribute to SLE pathogenesis, promoting both the production of anti-dsDNA antibodies and elevated interferon production. However, in addition to serving as a receptor for SEMA4D, CD72 seems to exert other functions. For example, B cell surface CD72 functions as a ligand for CD5 and induces T cell proliferation[77]. In addition, CD72 recognizes the endogenous TLR7 ligand Sm/ribonucleoprotein (RNP) and inhibits B cell production of anti-Sm/RNP antibodies, which are antibodies associated with SLE development[78]. Thus, the potential function of CD72 in SLE development could reflect its semaphorin-dependent and/or semaphorin-independent functions.

Systemic sclerosis

SSc is characterized by thickening of the skin and injuries of the microvasculature. The immune system is thought to contribute to some of the clinical and pathological manifestations of SSc, but the pathogenesis of this disease has not been completely elucidated.

SEMA4D expression in $CD4^+$ T cells and serum levels of soluble SEMA4D are elevated in patients with SSc compared with healthy individuals[39]. Although the cellular source of soluble SEMA4D is not clear, dysregulated

Table 2 | **Involvement of immune semaphorins-associated molecules in the pathogenesis of autoimmune rheumatic diseases**

Ligands	Ligand-expressing cells	Binding partners	Binding partner-expressing cells	Functions	Diagnostic relevance	Therapeutic relevance	Refs
Systemic lupus erythematous							
SEMA3A	Not defined	Not defined	B cells	Inhibition of TLR9-mediated B cell responses	Low serum SEMA3A levels in patients with SLE	Not shown	34
Not defined	Not defined	Nrp1	Cells in glomerular tissue	Not defined	High NRP1 expressions in glomeruli of patients with SLE	Not shown	33
SEMA4D	T cells?	CD72	B cells	Class switching of B cells	Low CD72 expressions in B cells of patients with SLE (B cells)	Not shown	32
Not defined	Not defined	Soluble CD72	Not defined	Not defined	High soluble CD72 serum levels in patients with SLE	Not shown	36
Sm and RNP proteins	Apoptotic cells	CD72	B cells	Inhibition of B cell response	Not shown	Not shown	78
Systemic sclerosis							
SEMA3A	T_{reg} cells	Not defined	Not defined	Reduces suppressive activity of T_{reg} cells	Low SEMA3A serum levels in patients with systemic sclerosis	Not shown	40
SEMA3E	Skin vascular endothelial cells	Plexin D1	Skin vascular endothelial cells	Promotes antiangiogenic effect	High SEMA3E serum levels in patients with systemic sclerosis	SEMA3E–Plexin D1 complex peptide	43
SEMA4A	Lung fibroblasts	Plexin D1	Lung fibroblasts	Promotes contraction of fibroblasts	Not shown	Anti-AKT antibody	54
SEMA4D	$CD4^+$ T cells?	Not defined	Not defined	Not defined	High SEMA4D serum levels in patients with systemic sclerosis	Not shown	39
SEMA7A	Fibrocytes and $CD19^+$ lymphocytes	β1 integrin	$CD14^+$ monocytes	Promotes differentiation of fibrocytes	Not shown	β1 integrin neutralization	38
Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis							
SEMA4D (as a receptor; reverse signalling)	Neutrophils	Plexin B2 (as a ligand)	Endothelial cells	Inhibits neutrophil activation	High Sema4D serum levels in patients with AAV	Not shown	41
SEMA6A*	Not defined	Not defined	Not defined	Not defined	Not shown	Not shown	42

*GWAS study. AAV. anti-neutrophil cytoplasmic antibody-associated vasculitis; NRP1, neuropilin 1; RNP, ribonucleoprotein; SEMA3A, semaphorin 3A; SLE, systemic lupus erythematosus; TLR9; Toll-like receptor 9.

SEMA4D expression and cleavage of this molecule is associated with serum levels of anti-Scl70 antibody, disease type (diffuse or limited cutaneous SSc), thickening of skin and disease duration[39]. Therefore, dysregulation of SEMA4D expression and cleavage of this molecule might have a role in the development and maintenance of SSc[39].

SEMA7A is expressed by mouse and human collagen-producing fibrocytes and $CD19^+$ lymphocytes, and contributes to the development of lung fibrosis associated with SSc[38]. Transgenic mice overexpressing TGFβ1 are prone to pulmonary fibrosis. Knock-out of *Sema7a* in these transgenic mice decreases the likelihood and severity of lung fibrosis and reduces the extent of alveolar remodelling. Peripheral blood mononuclear cells from patients with SSc-related interstitial lung disease have elevated levels of *SEMA7A* mRNA compared with healthy individuals. Recombinant SEMA7A induces the differentiation of normal human peripheral blood mononuclear cells into fibrocytes. This effect is attenuated by β1 integrin neutralization *in vitro*, which also attenuates pulmonary fibrosis in TGF- β1 transgenic mice[38]. Thus, prevention of the interaction between SEMA7A and β1 integrin represents a promising strategy for treating TGFβ1–driven or fibrocyte-associated autoimmune fibrosis.

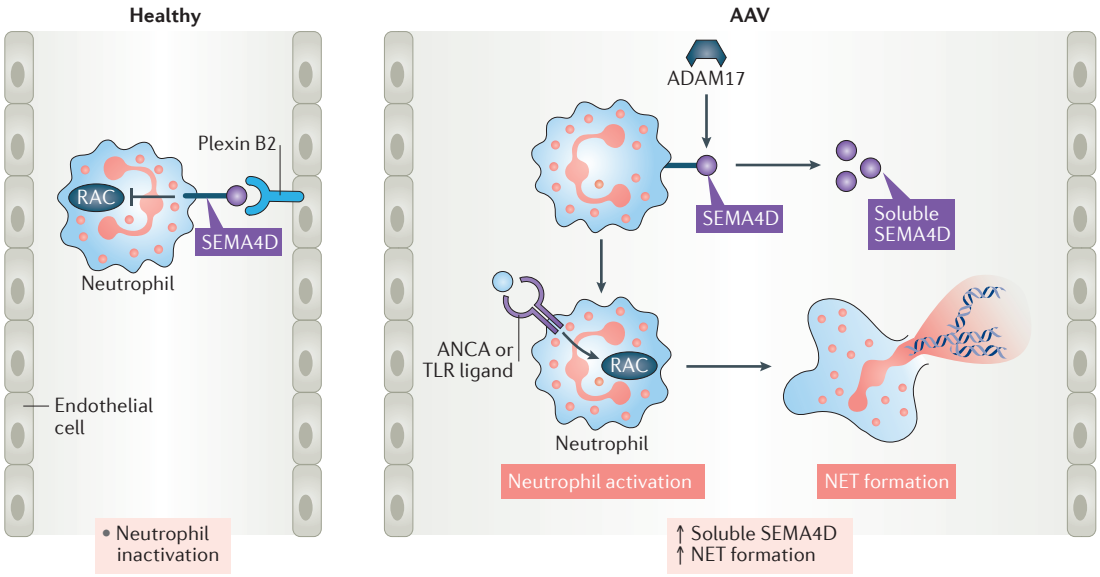

Figure 4 | **Involvement of SEMA4D in the pathogenesis of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis.** In healthy conditions, the interaction between the plexin B2 ligand on endothelial cells and the SEMA4D receptor on the surface of neutrophils inhibits RAS-related C3 botulinum toxin substrate (RAC1) activation in neutrophils and negatively regulates neutrophil activation. By contrast, in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), SEMA4D is proteolytically cleaved from the surface of neutrophils. Alterations in SEMA4D–plexin B2 interactions results in inappropriate activation of neutrophils such as Toll-like receptor (TLR) ligand-induced or ANCA-induced neutrophil extracellular trap (NET) formation, and is involved in the pathogenesis of AAV. ADAM17, disintegrin and metalloproteinase 17.

Serum SEMA3E levels are substantially higher in patients with either primary Raynaud phenomenon or SSc in comparison with healthy individuals[43]. Among patients with SSc, SEMA3E levels are considerably higher in those who have an early-phase nailfold video-capillaroscopy pattern than in those with active or late-phase patterns. In addition, patients with SSc without digital ulcers have higher serum SEMA3E levels than patients with ulcers, suggesting that increased serum levels of Sema3E are associated with early vascular involvement in SSc. SEMA3E is strongly upregulated in the skin microvascular endothelium in patients with SSc. *In vitro* stimulation of healthy microvascular endothelial cells with sera from patients with SSc increases the levels of both phosphorylated plexin D1 and SEMA3E in these cells, promoting an antiangiogenic effect. The addition of a SEMA3E–Plexin D1 combined peptide attenuates the antiangiogenic effect of SSc sera on endothelial cells[43]. Together, these data suggest that aberrant SEMA3E expression in the endothelium in SSc might have a role in the dysregulation of angiogenesis and neurovascular alterations of this disease, which is particularly clinically evident in the early phases of disease.

SEMA3A has also been implicated in the pathogenesis of SSc, mainly by affecting the activation of T_{reg} cells[40]. Serum levels of SEMA3A in patients with SSc are similar to the levels observed in patients with SLE, and lower than those observed in healthy individuals. Serum levels of SEMA3A inversely correlate with the duration of disease and positively correlate with the levels of anti–Scl-70 antibody. The expression of SEMA3A in T_{reg} cells is also lower in patients with SSc than in healthy individuals, which might explain the reduced activation of T_{reg} cells in SSc. Further studies are needed to confirm how the reduced activation of T_{reg} cells contribute to in pathogenesis of SSc.

Hence, secreted SEMA3A and SEMA4D have been implicated in SSc pathogenesis, via the induction of autoimmune responses. In addition, SEMA3E and SEMA7A are associated with specific symptoms of SSc including microvascular ischaemia and organ fibrosis.

ANCA-associated vasculitis

Several reports have demonstrated a relationship between semaphorins and ANCA-associated vasculitis (AAV). In 2013, Xie *et al.* carried out a genome-wide association study (GWAS) of 492 patients with granulomatosis with polyangiitis (GPA) and 1,506 healthy controls (white individuals of European descent), followed by a replication analysis of the most strongly associated signals in an independent cohort of 528 individuals with GPA and 1,228 healthy controls[42]. In this GWAS, the investigators identified the *SEMA6A* locus as an important contributor to the risk of developing GPA. In addition, an independent single-nucleotide polymorphism, rs26595, near the *SEMA6A* gene on chromosome 5, was associated with GPA, reaching genome-wide significance in a combined analysis of the GWAS and replication cohorts ($P = 2.09 \times 10^{-8}$). However, in 2014 Wieczorek *et al.* commented that the differences in allele frequencies between the combined cohorts and the respective subgroups from

Germany, Netherlands and the UK were not statistically significant[79]. Therefore, further genetic association studies on GPA are needed to confirm the associations observed in these studies.

In 2017, a unique function of SEMA4D in neutrophils and its pathological involvement in AAV was reported[41]. Neutrophil extracellular trap (NET) formation is enhanced in patients with AAV, and this inflammatory response from neutrophils and the interaction of these cells with small vessel endothelium have important pathological roles in AAV. SEMA4D was shown to inhibit neutrophil activation by functioning as a receptor for endothelial plexin B2, the disruption of which has been implicated in the pathogenesis of AAV. Serum levels of soluble SEMA4D are elevated in patients with AAV, owing to ADAM17-mediated cleavage of SEMA4D from the surface of activated neutrophils, and correlates with clinical disease scores. SEMA4D on the neutrophil cell surface binds to plexin B2 on endothelial cells, and this binding is required for the suppression of NET formation. This suppression is impaired when plexin B2 on endothelial cells is knocked down by short-hairpin RNA. Furthermore, treating neutrophils with recombinant plexin B2 considerably inhibits the neutrophil oxidative burst by suppressing RAC1 activation. Deletion of the cytoplasmic tail of SEMA4D disrupts this inhibitory effect, which suggests that the SEMA4D receptor on neutrophil interacts with the plexin B2 ligand on endothelial cells, thereby blocking inflammation[41]. Hence, close proximity of neutrophils to the endothelium in narrow blood vessels should decrease the probability that the cells are inappropriately activated. When ADAM17 is active, as in AAV[41,80], this enzyme cleaves SEMA4D on neutrophils from the membrane. The resultant soluble SEMA4D promotes inflammatory effects on endothelial cells. Furthermore, disruption of the SEMA4D–plexin B2 interaction causes abnormal activation of neutrophils, which contributes to AAV pathogenesis (FIG. 4). Consequently, soluble SEMA4D in serum could be used as a diagnostic marker for AAV. Moreover, membrane-bound SEMA4D functions as a regulator of neutrophil activation, raising the possibility that SEMA4D could also serve as a target for therapies aimed at clinically managing autoimmune vasculitis mediated by neutrophils.

Conclusions

The immune semaphorins are critical regulators of many aspects of immunity. A large body of literature has shown that disruption or modification of semaphorin signalling has a causative role in immune disorders, including autoimmune diseases. Based on the increasing knowledge of the molecular mechanisms of immune regulation by semaphorins and their interacting factors, we predict that these proteins have therapeutic potential in the context of immunological diseases, including autoimmune rheumatic diseases. As mentioned, multiple studies have implicated semaphorins as therapeutic targets to protect against dysregulated autoimmune responses in RA, SLE, SSc and AAV. Ongoing clinical trials are investigating drugs that target particular immune semaphorins or receptors such as SEMA4D and NRP1. Before the clinical application of these drugs, however, it will be necessary to assess the nature and severity of any off-target effects, including in tissues and organs such as the brain, spinal cord and vasculature. It is also important to note that NRPs also interact with ligands other than semaphorins. Moving towards the goal of identifying and validating targets for treating autoimmune disorders, the properties of these molecules should be comprehensively elucidated both *in vitro* (for example, subjecting recombinant proteins to quantitative binding assays) and *in vivo* (for example, using genetically modified mouse models).

1. Kolodkin, A. L., Matthes, D. J. & Goodman, C. S. The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. *Cell* **75**, 1389–1399 (1993).
2. Toyofuku, T. *et al.* Guidance of myocardial patterning in cardiac development by Sema6D reverse signalling. *Nat. Cell Biol.* **6**, 1204–1211 (2004).
3. Gu, C. *et al.* Semaphorin 3E and plexin-D1 control vascular pattern independently of neuropilins. *Science* **307**, 265–268 (2005).
4. Casazza, A. *et al.* Sema3E-Plexin D1 signaling drives human cancer cell invasiveness and metastatic spreading in mice. *J. Clin. Invest.* **120**, 2684–2698 (2010).
5. Maione, F. *et al.* Semaphorin 3A is an endogenous angiogenesis inhibitor that blocks tumor growth and normalizes tumor vasculature in transgenic mouse models. *J. Clin. Invest.* **119**, 3356–3372 (2009).
6. Maione, F. *et al.* Semaphorin 3A overcomes cancer hypoxia and metastatic dissemination induced by antiangiogenic treatment in mice. *J. Clin. Invest.* **122**, 1832–1848 (2012).
7. Negishi-Koga, T. *et al.* Suppression of bone formation by osteoclastic expression of semaphorin 4D. *Nat. Med.* **17**, 1473–1480 (2011).
8. Takegahara, N. *et al.* Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. *Nat. Cell Biol.* **8**, 615–622 (2006).
9. Hayashi, M. *et al.* Osteoprotection by semaphorin 3A. *Nature* **485**, 69–74 (2012).
10. Toyofuku, T. *et al.* Endosomal sorting by Semaphorin 4A in retinal pigment epithelium supports photoreceptor survival. *Genes Dev.* **26**, 816–829 (2012).
11. Nojima, S. *et al.* A point mutation in Semaphorin 4A associates with defective endosomal sorting and causes retinal degeneration. *Nat. Commun.* **4**, 1406 (2013).
12. Bougeret, C. *et al.* Increased surface expression of a newly identified 150-kDa dimer early after human T lymphocyte activation. *J. Immunol.* **148**, 318–323 (1992).
13. Kumanogoh, A. *et al.* Identification of CD72 as a lymphocyte receptor for the class IV semaphorin CD100: a novel mechanism for regulating B cell signaling. *Immunity* **13**, 621–631 (2000).
14. Shi, W. *et al.* The class IV semaphorin CD100 plays nonredundant roles in the immune system: defective B and T cell activation in CD100-deficient mice. *Immunity* **13**, 633–642 (2000).
15. Kumanogoh, A. *et al.* Class IV semaphorin Sema4A enhances T-cell activation and interacts with Tim-2. *Nature* **419**, 629–633 (2002).
16. Kumanogoh, A. *et al.* Nonredundant roles of Sema4A in the immune system: defective T cell priming and Th1/Th2 regulation in Sema4A-deficient mice. *Immunity* **22**, 305–316 (2005).
17. Suzuki, K. *et al.* Semaphorin 7A initiates T-cell-mediated inflammatory responses through α1β1 integrin. *Nature* **446**, 680–684 (2007).
18. Takamatsu, H. *et al.* Semaphorins guide the entry of dendritic cells into the lymphatics by activating myosin II. *Nat. Immunol.* **11**, 594–600 (2010).
19. Suzuki, K., Kumanogoh, A. & Kikutani, H. Semaphorins and their receptors in immune cell interactions. *Nat. Immunol.* **9**, 17–23 (2008).
20. Kumanogoh, A. & Kikutani, H. Immunological functions of the neuropilins and plexins as receptors for semaphorins. *Nat. Rev. Immunol.* **13**, 802–814 (2013).
21. Pasterkamp, R. J. Getting neural circuits into shape with semaphorins. *Nat. Rev. Neurosci.* **13**, 605–618 (2012).
22. Takamatsu, H. & Kumanogoh, A. Diverse roles for semaphorin-plexin signaling in the immune system. *Trends Immunol.* **33**, 127–135 (2012).
23. Tamagnone, L. Emerging role of semaphorins as major regulatory signals and potential therapeutic targets in cancer. *Cancer Cell* **22**, 145–152 (2012).
24. Miller, L. E. *et al.* Increased prevalence of semaphorin 3C, a repellent of sympathetic nerve fibers, in the synovial tissue of patients with rheumatoid arthritis. *Arthritis Rheum.* **50**, 1156–1163 (2004).
25. Catalano, A. The neuroimmune semaphorin-3A reduces inflammation and progression of experimental autoimmune arthritis. *J. Immunol.* **185**, 6373–6383 (2010).
26. Takagawa, S. *et al.* Decreased semaphorin3A expression correlates with disease activity and histological features of rheumatoid arthritis. *BMC Musculoskelet. Disord.* **14**, 40 (2013).
27. Gras, C. *et al.* Secreted semaphorin 5A activates immune effector cells and is a biomarker for rheumatoid arthritis. *Arthritis Rheumatol.* **66**, 1461–1471 (2014).

28. Chapoval, S. P. Semaphorin 4A as novel regulator and promising therapeutic target in rheumatoid arthritis. *Arthritis Res. Ther.* **17**, 313 (2015).
29. Wang, L. *et al.* Expression of Semaphorin 4A and its potential role in rheumatoid arthritis. *Arthritis Res. Ther.* **17**, 227 (2015).
30. Yoshida, Y. *et al.* Semaphorin 4D contributes to rheumatoid arthritis by inducing inflammatory cytokine production: pathogenic and therapeutic implications. *Arthritis Rheumatol* **67**, 1481–1490 (2015).
31. Xie, J. & Wang, H. Semaphorin 7A as a potential immune regulator and promising therapeutic target in rheumatoid arthritis. *Arthritis Res. Ther.* **19**, 10 (2017).
32. Nakano, S. *et al.* Down-regulation of CD72 and increased surface IgG on B cells in patients with lupus nephritis. *Autoimmunity* **40**, 9–15 (2007).
33. Vadasz, Z. *et al.* The involvement of immune semaphorins and neuropilin-1 in lupus nephritis. *Lupus* **20**, 1466–1473 (2011).
34. Vadasz, Z. & Toubi, E. Semaphorin 3A — a marker for disease activity and a potential putative disease-modifying treatment in systemic lupus erythematosus. *Lupus* **21**, 1266–1270 (2012).
35. Vadasz, Z. *et al.* A regulatory role for CD72 expression on B cells in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **43**, 767–771 (2014).
36. Vadasz, Z. *et al.* Increased soluble CD72 in systemic lupus erythematosus is in association with disease activity and lupus nephritis. *Clin. Immunol.* **164**, 114–118 (2016).
37. Gao, H. *et al.* Expression and clinical significance of Semaphorin 3A in serum and mononuclear cells in patients with systemic lupus erythematosus [Chinese]. *Zhonghua Yi Xue Za Zhi* **97**, 370–374 (2017).
38. Gan, Y. *et al.* Role of semaphorin 7a signaling in transforming growth factor β1-induced lung fibrosis and scleroderma-related interstitial lung disease. *Arthritis Rheum.* **63**, 2484–2494 (2011).
39. Besliu, A. *et al.* Peripheral blood lymphocytes analysis detects CD100/SEMA4D alteration in systemic sclerosis patients. *Autoimmunity* **44**, 427–436 (2011).
40. Rimar, D. *et al.* Semaphorin 3A: an immunoregulator in systemic sclerosis. *Rheumatol. Int.* **35**, 1625–1630 (2015).
41. Nishide, M. *et al.* Semaphorin 4D inhibits neutrophil activation and is involved in the pathogenesis of neutrophil-mediated autoimmune vasculitis. *Ann. Rheum. Dis.* **76**, 1440–1448 (2017).
42. Xie, G. *et al.* Association of granulomatosis with polyangiitis (Wegener's) with *HLA-DPB1*04* and *SEMA6A* gene variants: evidence from genome-wide analysis. *Arthritis Rheum.* **65**, 2457–2468 (2013).
43. Mazzotta, C. *et al.* Plexin-D1/Semaphorin 3E pathway may contribute to dysregulation of vascular tone control and defective angiogenesis in systemic sclerosis. *Arthritis Res. Ther.* **17**, 221 (2015).
44. Catalano, A. *et al.* Semaphorin-3A is expressed by tumor cells and alters T-cell signal transduction and function. *Blood* **107**, 3321–3329 (2006).
45. Yamamoto, M. *et al.* Plexin-A4 negatively regulates T lymphocyte responses. *Int. Immunol.* **20**, 413–420 (2008).
46. Wen, H., Lei, Y., Eun, S. Y. & Ting, J. P. Plexin-A4-semaphorin 3A signaling is required for Toll-like receptor- and sepsis-induced cytokine storm. *J. Exp. Med.* **207**, 2943–2957 (2010).
47. Yamashita, N. *et al.* Anti-Semaphorin 3A neutralization monoclonal antibody prevents sepsis development in lipopolysaccharide-treated mice. *Int. Immunol.* **27**, 459–466 (2015).
48. Choi, Y. I. *et al.* PlexinD1 glycoprotein controls migration of positively selected thymocytes into the medulla. *Immunity* **29**, 888–898 (2008).
49. Movassagh, H., Shan, L., Mohammed, A., Halayko, A. J. & Gounni, A. S. Semaphorin 3E deficiency exacerbates airway inflammation, hyperresponsiveness, and remodeling in a mouse model of allergic asthma. *J. Immunol.* **198**, 1805–1814 (2017).
50. Movassagh, H. *et al.* Semaphorin 3E alleviates hallmarks of house dust mite-induced allergic airway disease. *Am. J. Pathol.* **187**, 1566–1576 (2017).
51. Tamagnone, L. *et al.* Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. *Cell* **99**, 71–80 (1999).
52. Witherden, D. A. *et al.* The CD100 receptor interacts with its plexin B2 ligand to regulate epidermal γδ T cell function. *Immunity* **37**, 314–325 (2012).
53. Delgoffe, G. M. *et al.* Stability and function of regulatory T cells is maintained by a neuropilin-1-semaphorin-4a axis. *Nature* **501**, 252–256 (2013).
54. Peng, H. Y., Gao, W., Chong, F. R., Liu, H. Y. & Zhang, J. I. Semaphorin 4A enhances lung fibrosis through activation of Akt via PlexinD1 receptor. *J. Biosci.* **40**, 855–862 (2015).
55. Toyofuku, T. *et al.* Semaphorin-4A, an activator for T-cell-mediated immunity, suppresses angiogenesis via Plexin-D1. *EMBO J.* **26**, 1373–1384 (2007).
56. Rennert, P. D. *et al.* T cell, Ig domain, mucin domain-2 gene-deficient mice reveal a novel mechanism for the regulation of Th2 immune responses and airway inflammation. *J. Immunol.* **177**, 4311–4321 (2006).
57. Mogie, G. *et al.* Neuroimmune semaphorin 4A as a drug and drug target for asthma. *Int. Immunopharmacol.* **17**, 568–575 (2013).
58. Nakagawa, Y. *et al.* Identification of semaphorin 4B as a negative regulator of basophil-mediated immune responses. *J. Immunol.* **186**, 2881–2888 (2011).
59. Ishida, I. *et al.* Involvement of CD100, a lymphocyte semaphorin, in the activation of the human immune system via CD72: implications for the regulation of immune and inflammatory responses. *Int. Immunol.* **15**, 1027–1034 (2003).
60. Oinuma, I., Ishikawa, Y., Katoh, H. & Negishi, M. The Semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. *Science* **305**, 862–865 (2004).
61. Kumanogoh, A. *et al.* Requirement for CD100-CD72 interactions in fine-tuning of B-cell antigen receptor signaling and homeostatic maintenance of the B-cell compartment. *Int. Immunol.* **17**, 1277–1282 (2005).
62. Hall, K. T. *et al.* Human CD100, a novel leukocyte semaphorin that promotes B-cell aggregation and differentiation. *Proc. Natl Acad. Sci. USA* **93**, 11780–11785 (1996).
63. Granziero, L. *et al.* CD100/Plexin-B1 interactions sustain proliferation and survival of normal and leukemic $CD5^+$ B lymphocytes. *Blood* **101**, 1962–1969 (2003).
64. Kumanogoh, A. *et al.* Requirement for the lymphocyte semaphorin, CD100, in the induction of antigen-specific T cells and the maturation of dendritic cells. *J. Immunol.* **169**, 1175–1181 (2002).
65. Kataoka, T. R., Kumanogoh, A., Bandara, G., Metcalfe, D. D. & Gilfillan, A. M. CD72 negatively regulates KIT-mediated responses in human mast cells. *J. Immunol.* **184**, 2468–2475 (2010).
66. Goldberg, J. L. *et al.* An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. *J. Neurosci.* **24**, 4989–4999 (2004).
67. Sadanandam, A., Rosenbaugh, E. G., Singh, S., Varney, M. & Singh, R. K. Semaphorin 5A promotes angiogenesis by increasing endothelial cell proliferation, migration, and decreasing apoptosis. *Microvasc. Res.* **79**, 1–9 (2010).
68. Matsuoka, R. L. *et al.* Class 5 transmembrane semaphorins control selective Mammalian retinal lamination and function. *Neuron* **71**, 460–473 (2011).
69. Artigiani, S. *et al.* Plexin-B3 is a functional receptor for semaphorin 5A. *EMBO Rep.* **5**, 710–714 (2004).
70. O'Connor, B. P. *et al.* Semaphorin 6D regulates the late phase of $CD4^+$ T cell primary immune responses. *Proc. Natl Acad. Sci. USA* **105**, 13015–13020 (2008).
71. Pasterkamp, R. J., Peschon, J. J., Spriggs, M. K. & Kolodkin, A. L. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature* **424**, 398–405 (2003).
72. Kong, J. S. *et al.* Anti-neuropilin-1 peptide inhibition of synoviocyte survival, angiogenesis, and experimental arthritis. *Arthritis Rheum.* **62**, 179–190 (2010).
73. Patnaik, A. *et al.* Safety, pharmacokinetics, and pharmacodynamics of a humanized anti-Semaphorin 4D antibody, in a first-in-human study of patients with advanced solid tumors. *Clin. Cancer Res.* **22**, 827–836 (2016).
74. LaGanke, C. *et al.* Safety/tolerability of the anti-semaphorin 4D antibody VX15/2503 in a randomized phase 1 trial. *Neurol. Neuroimmunol. Neuroinflamm* **4**, e367 (2017).
75. Sierra, J. R. *et al.* Tumor angiogenesis and progression are enhanced by Sema4D produced by tumor-associated macrophages. *J. Exp. Med.* **205**, 1673–1685 (2008).
76. Evans, E. E., Paris, M., Smith, E. S. & Zauderer, M. Immunomodulation of the tumor microenvironment by neutralization of Semaphorin 4D. *Oncoimmunology* **4**, e1054599 (2015).
77. Van de Velde, H., von Hoegen, I., Luo, W., Parnes, J. R. & Thielemans, K. The B-cell surface protein CD72/Lyb-2 is the ligand for CD5. *Nature* **351**, 662–665 (1991).
78. Akatsu, C. *et al.* CD72 negatively regulates B lymphocyte responses to the lupus-related endogenous toll-like receptor 7 ligand Sm/RNP. *J. Exp. Med.* **213**, 2691–2706 (2016).
79. Wieczorek, S. *et al.* The SEM6A6 locus is not associated with granulomatosis with polyangiitis or other forms of antineutrophil cytoplasmic antibody-associated vasculitides in Europeans: comment on the article by Xie *et al. Arthritis Rheumatol.* **66**, 1400–1401 (2014).
80. Bertram, A. *et al.* Circulating ADAM17 level reflects disease activity in proteinase-3 ANCA-associated vasculitis. *J. Am. Soc. Nephrol.* **26**, 2860–2870 (2015).

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Preventing progression from arthralgia to arthritis: targeting the right patients

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Abstract | Early treatment is associated with improved outcomes in patients with rheumatoid arthritis (RA), suggesting that a 'window of opportunity', in which the disease is most susceptible to disease-modifying treatment, exists. Autoantibodies and markers of systemic inflammation can be present long before clinical arthritis, and maturation of the immune response seems to coincide with the development of RA. The pre-arthritis phase associated with symptoms such as as joint pain without clinical arthritis (athralgia) is now hypothesized to fall within the aforementioned window of opportunity. Consequently, disease modulation in this phase might prevent the occurrence of clinically apparent arthritis, which would result in a persistent disease course if untreated. Several ongoing proof-of-concept trials are now testing this hypothesis. This Review highlights the importance of adequate risk prediction for the correct design, execution and interpretation of results of these prevention trials, as well as considerations when translating these findings into clinical practice. The patients' perspectives are discussed, and the accuracy with which RA development can be predicted in patients presenting with arthralgia is evaluated. Currently, the best starting position for preventive studies is proposed to be the inclusion of patients with an increased risk of RA, such as those identified as fulfilling the EULAR definition of 'arthralgia suspicious for progression to RA'.

Early initiation of effective DMARDs and the treat-to-target approach are the cornerstones of current treatment strategies for rheumatoid arthritis (RA)[1,2]. Underlying the relevance of early treatment initiation is the concept of a 'window of opportunity', which presumes that a confined period exists in which the disease is most susceptible to the disease-modifying effects of treatment[3,4]. Although the exact timeline of disease progression has yet to be determined, an important proportion of this window could be situated before arthritis becomes clinically evident.

Current therapies for RA are effective in suppressing inflammation, but their ability to modify disease persistence is limited[5]. Retrospective nested case–control studies have revealed that RA-related autoantibodies and markers of systemic or local subclinical inflammation can be present months or years before diagnosis[6–12], demonstrating that the disease process is evolving long before the disease becomes clinically detectable. On the basis of current understanding of RA aetiopathogenesis, the EULAR study group for risk factors for RA has defined several phases of RA development according to the presence of particular features: genetic and environmental risk factors for RA; autoimmunity associated with RA; symptoms such as joint pain but without clinical arthritis (arthralgia); and clinical arthritis (which can be either unclassified arthritis or RA)[13]. Such observations have encouraged a call for 'preventive trials'; that is, trials that assess treatment initiation in pre-arthritis phases with the ultimate aim of preventing the onset of RA (FIG. 1).

The challenge of RA prevention raises questions concerning how to accurately identify individuals in the pre-arthritis phases, how to avoid overtreatment and how to manage patients that are presumed to be at risk of developing RA. In this Review, we discuss what is known about the identification of patients at risk of developing RA in different pre-arthritis phases, particularly patients with arthralgia, and the methodological concerns of designing clinical trials that include such patients.

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Key points

- Early treatment initiation in patients with clinically manifest rheumatoid arthritis (RA) is associated with improved disease outcomes; hence, disease modulation in pre-arthritis phases might prevent the occurrence of clinical arthritis
- The inclusion of patients with a low risk of developing RA might dilute possible preventive effects and result in false-negative results in preventive trials
- Although a symptomatic phase typically precedes clinical arthritis in patients who develop RA, arthralgia is common and is not specific enough to identify patients at risk of developing RA
- The EULAR definition of 'arthralgia suspicious for progression to RA', which identifies patients with arthralgia at risk of developing RA, is a good starting position for preventive trial participant selection
- Adequate stratification of patients with arthralgia at risk of developing RA requires a combination of clinical, serological and imaging markers

Research into preventive treatment

Efficacy of early treatment. At present, all evidence supporting early treatment initiation come from studies of patients with clinically manifest arthritis[2,14]. Very few trials on treatment initiated in the pre-arthritis phases have been published to date.

Results from studies in experimental animal models of arthritis suggest that providing treatment before arthritis is clinically evident is efficacious. In 2017, a systematic literature review[15], which included a meta-analysis of 16 such animal model studies, demonstrated that starting immunosuppressive treatment in the induction phase of experimental arthritis (that is, before the development of clinical arthritis and the autoantibody response), has beneficial effects on arthritis severity compared with no treatment. Data was most compelling for methotrexate and abatacept (an inhibitor of T cell co-stimulation). In mice that had autoantibodies but no clinical arthritis, representing a setting in which autoimmunity has developed but not yet clinical arthritis, treatment was also effective. Methotrexate seemed to be more effective than TNF inhibition in this setting, although the different medications were not directly compared in any of the studies included in the meta-analysis[15]. Among the numerous limitations of these experimental studies, two are especially relevant when considering preventive treatment: first, the treatment period in most experiments was extended into the clinical phase and not confined to the pre-arthritis phase, and second, the outcome was arthritis severity and not the development of clinically detectable arthritis. So, although the trends in these animal studies favour the relevance of pre-arthritis treatment, larger studies with treatment confined to the pre-arthritis phase and with head-to-head comparisons of different treatments, such as methotrexate versus abatacept, will yield more information on the preventive effects of DMARDs in experimental models.

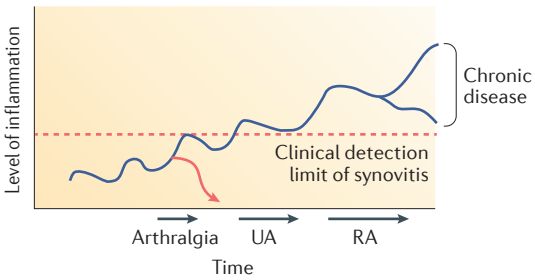

Figure 1 | **Rheumatoid arthritis development over time in relation to the level of inflammation.** Subclinical inflammation can progress to clinically detectable, persistent and classifiable rheumatoid arthritis (RA) (indicated by the blue line). The initiation of disease-modifying treatment in patients with arthralgia (that is, patients in the pre-arthritis phase who have symptoms such as joint point) might prevent disease development (indicated by the red line).The red dotted line indicates the clinical detection limit for measuring inflammation (thus, the transition from subclinical inflammation to clinically detectable inflammation) . This figure was adapted with permission obtained from van der Helm-van Mil, A. *Nat. Rev. Rheumatol.* 10, 171–180 (2014). UA, undifferentiated arthritis.

The first placebo-controlled trial assessing the initiation of treatment in pre-arthritis in humans was published in 2009 and demonstrated that two intramuscular injections of dexamethasone in seropositive patients with arthralgia decreased autoantibody levels, but did not prevent the development of arthritis[16]. In 2016, results from the PRAIRI (prevention of clinically manifest RA by B cell directed therapy in the earliest phase of the disease) trial demonstrated that a single infusion of rituximab in seropositive patients with arthralgia and any sign of systemic and/or local inflammation delayed, but did not prevent, the development of clinical arthritis[17] (TABLE 1). Several other proof-of-concept trials are ongoing (TABLE 2). The study populations and the drugs used vary in the different trials, but in the majority of the trials the presence of RA-related autoantibodies (an indicator of RA-associated autoimmunity) is an inclusion criterion. Publication of the results from these trials over the next decade will increase our understanding of whether such interventions can effectively prevent chronic arthritis and, if so, in which subsets of at-risk individuals.

Until positive results are obtained from any these proof-of-concept studies, no evidence is available to support the use of DMARDs in patients without clinical arthritis, which is in line with published recommendations[1,2]. However, as such patients might already be experiencing pain and functional limitations, prescribing NSAIDs or other pain killers to reduce pain seems logical, as is close monitoring of these patients for the development of clinical arthritis.

The importance of risk stratification. Risk stratification is an essential strategy for advancing research in RA prevention. Adequate risk stratification is crucial when designing and interpreting the results of preventive studies; within the study population, the risk each individual has of developing the disease outcome (such as clinically evident RA) considerably affects the power of the study. The greater the percentage of individuals included in the study that have a low risk of developing RA within 1 or 2 years (known as 'non-informative' inclusions),

Table 1 | **Completed proof-of-concept treatment studies in patients with arthralgia**

Ref	Year of publication	No. of subjects	Participants	Intervention	Outcome measure(s)	Follow-up duration	Outcome
Bos *et al.*[16]	2009	83	Patients with arthralgia who were either ACPA-positive or rheumatoid factor-positive, and had the presence of the shared epitope	Intramuscular injection of either dexamethasone (100 mg) or placebo at 0 and 6 weeks	Primary outcome: 50% reduction of ACPA or rheumatoid factor levels at 6 months; secondary outcome: the development of clinical arthritis	Median 26 months (IQR 21–37)	Arthritis development was similar in both groups (20% versus 21%). In each group 50% of patients had a reduction in one or both autoantibodies; in the intervention group, autoantibody levels significant decreased after 1 month (ACPA -22%, RF -14%), which persisted at 6 months for ACPA; in the placebo group no significant decreases in autoantibody levels were demonstrated.
Gerlag *et al.*[17]	2016*	82	Patients with arthralgia who are positive for both ACPAs and rheumatoid factor, and have CRP levels ≥3 mg/l and/or subclinical synovitis as detected by ultrasonography or MRI of the hands	Intravenous Rituximab (1000 mg) or placebo following intramuscular methylprednisolone (100 mg) premedication	Development of clinically manifest arthritis	Median 29 months (range 0– 54)	40% of patients developed arthritis in the placebo group after a median period of 11.5 months and 34% in patients in the rituximab group developed arthritis after a median period of 16.5 months, which represented a significant delay in the development of arthritis, but not a significant prevention of arthritis.

This table demonstrates the current absence of evidence for treating patients with arthralgia in order to prevent clinical arthritis. ACPA, anti-citrullinated peptide antibody; CRP, C-reactive protein; IQR, interquartile range. *Abstract publication (full article not yet published).

the lower the power of the study. This phenomenon is especially notable in trials with relatively low samples sizes, such as some of the preventive trials performed over the past decade[16,17]. The importance of risk stratification was illustrated in 2017 in a *post hoc* analysis of the PROMPT (probable RA: methotrexate versus placebo treatment) trial[18,19]. In this trial, patients with undifferentiated arthritis were randomly allocated to receive either methotrexate or placebo in order to either prevent the development of RA (the primary outcome) or achieve drug-free remission (the secondary outcome)[18]. Analysis of the whole cohort showed that methotrexate treatment neither prevented RA development nor resulted in drug-free remission. Initial *post hoc* analysis suggested, however, that methotrexate had a beneficial effect in anti-citrullinated protein antibody (ACPA)-positive patients but not in ACPA-negative patients. Although the ACPA-positive patients had a higher risk of developing RA than ACPA-negative patients, stratifying patients solely on the basis of ACPA status was too simplistic. Previous studies investigating the natural course of undifferentiated arthritis have shown that only one-third of these patients will develop RA, whereas the rest develop different diagnoses or go into spontaneous remission[20,21]. Hence, investigators subsequently developed and validated a model that predicts the risk of undifferentiated arthritis progressing to RA in an individual patient, taking into account data on clinical features, the presence of rheumatoid factors or ACPAs and levels of C-reactive protein (CRP)[22,23]. When repeating the analyses of the PROMPT trial considering only those patients predicted to have a high risk of RA by use of this model (>80% probability of progression to RA in the next year; referred to here as 'high-risk' patients), methotrexate was shown to prevent RA development (with an estimated number needed to treat of 2.2)[19].

The PROMPT trial was performed before the development of the 2010 ACR–EULAR classification criteria for RA[24]. Therefore, the secondary outcome, DMARD-free remission, is of importance as this outcome was independent of classification criteria. Interestingly, methotrexate treatment increased the proportion of high-risk patients who achieved DMARD-free remission after 5 years of follow-up (none (0%) of the 11 patients in the placebo group versus four (36%) of the 11 patients in the methotrexate group)[19]. Further stratification of these high-risk patients by ACPA status showed a preventive effect in both ACPA-positive and ACPA-negative patients, whereas no effect was observed in ACPA-positive or ACPA-negative patients at a lower risk of developing RA, indicating that these two latter groups contained predominantly non-informative inclusions. In other words, the previous conclusion that methotrexate might only work in ACPA-positive patients with undifferentiated arthritis was attributable to the fact that this group of patients included a higher proportion of high-risk patients than the group of ACPA-negative patients with undifferentiated arthritis. Altogether, these data highlight the importance of patient stratification: only when studying patients with a high risk of developing RA was the important preventive effect observed. These results are based on *post hoc* analyses with small numbers of patients, but they underline the relevance of adequate prognostication in prevention trials in order to avoid false-negative trial results.

Table 2 | **Summary of ongoing placebo controlled proof-of-concept trials in pre-arthritis phases (preventive trials)**

Trial name	Year of start	Planned sample size	Participants	Intervention	Primary outcome measure	Follow-up duration
APIPPRA[61]	2014	206	Patients with non-traumatic arthralgia who are autoantibody-positive (that is, are either positive for both rheumatoid factor and ACPAs or have high levels of ACPAs)	Abatacept (125 mg weekly by subcutaneous injection) over 12 months	Development of either clinical arthritis or RA	24 months
ARIAA[62]	2014	95	Patients with arthralgia who are positive for ACPAs and have subclinical inflammation in the dominant hand as detected by MRI.	Abatacept (125 mg weekly by subcutaneous injection) over 6 months	Improvement of inflammation	18 months
TREAT EARLIER[63]	2015	200	Patients with clinically suspect arthralgia and subclinical MRI-inflammation in the most painful hand and/or foot	Methylprednisolone (120 mg by a single intramuscular injection) and methotrexate (25 mg weekly) over 12 months	Development of clinically-detectable arthritis (≥2 involved joints and persisting for ≥4 weeks)	24 months
STAPRA[64]	2015	220	Auto-antibody positive patients (that is patients who are either positive for both rheumatoid factor and ACPAs or have high levels of ACPA	Atorvastatin (40 mg daily) over 36 months	Development of clinically detectable arthritis	48 months
StopRA[65]	2016	200	ACPA-positive individuals without inflammatory arthritis; these patients are either FDRs of patients with RA, individuals recruited at health-fairs or individuals recruited from rheumatology clinics	Hydroxychloroquine (200–400 mg daily) over 12 months	Development of clinically apparent RA	36 months

ACPA, anti-citrullinated peptide antibody; FDR, first-degree relative; RA, rheumatoid arthritis

Shared decision-making between physicians and patients requires the physician to adequately inform the patient about their risk of developing RA. In the past 2 years, qualitative studies have revealed that individuals at risk of developing RA have difficulty interpreting their probability of developing RA in the future when it is expressed as a percentage, and that they prefer to receive a 'yes' or 'no' answer to the question of whether they will develop RA[25,26]. This finding implies that, in discussions with patients in the pre-arthritis phase about whether to initiate treatment, the most appropriate risk-prediction tools to use are those with high positive and negative predictive values (that is, tests with a clear-cut readout).

Translating research into clinical practice also depends on appropriate risk stratification. If the ongoing proof-of-concept studies (TABLE 2) are successful and their results support the treatment of patients with arthralgia in order to prevent clinically apparent arthritis, the next question will concern whom to treat. Insufficient risk stratification of proof-of-concept trial results might result in overtreatment, as patients that are only considered at low risk of developing RA would receive treatment. This overtreatment is highly undesirable, both from the perspective of individual patients and from the socio-economic point of view. Thus, adequate risk stratification is crucial.

Perceptions of preventive treatment. Interpreting and communicating with patients the risks and benefits of a treatment strategy is complicated, particularly in the setting of preventive trials, as not only is the efficacy and safety of a particular treatment strategy uncertain, so is the baseline risk of the patient population developing RA. Therefore, studies evaluating patient perceptions should include a multidisciplinary team of patients, health professionals and rheumatologists.

The importance of this communication is illustrated by the results of one trial investigating the benefits of personalized risk education; in this trial, those individuals at risk of RA who received personalized risk education, which incorporated factors such as smoking, diet, exercise and dental hygiene, were more motivated to change their health behaviours than individuals who received standard education about RA[27].

A patient's perception of the risks and benefits of preventive treatment can affect their willingness to take such medication. As mentioned above, individuals prefer a yes or no answer on the question of whether they will develop RA[24–26]. In 2016, a Swiss study evaluated, from the perspective of individuals at risk of developing RA (that is, 32 asymptomatic first-degree relatives (FDR) of patients with RA), what level of risk justifies the initiation of treatment, and which factors influence this decision[28]. Initially, the investigators assigned all participants a hypothetical baseline risk of developing RA. The participants were then presented with hypothetical scenarios, involving potential preventive treatments with a number of attributes of different levels (extent of risk reduction, risk of mild and serious adverse events and mode of administration), and were asked whether they would be willing to take the preventive treatment. Overall, the willingness to take preventive medication increased in parallel with the risk of developing RA: 38% of the FDRs studied would be willing to take medication

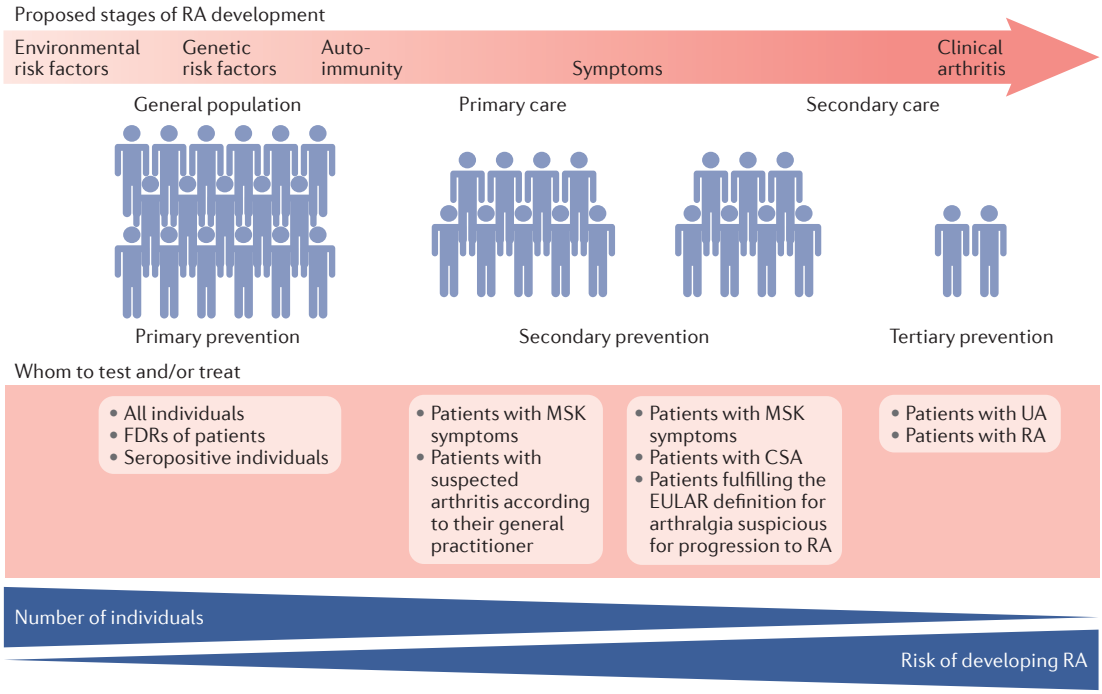

Figure 2 | **Different approaches for identifying individuals at risk of developing rheumatoid arthritis.** This figure presents the different phases of rheumatoid arthritis (RA) development proposed by the EULAR study group[13], in relation to the possible types of prevention (primary, secondary or tertiary). In addition, it illustrates the risk of RA development for different groups of individuals identified in different settings, and the relative sizes of these groups. Not all patients will pass through every phase of RA development and some phases can be present at the same time (for example, smoking, autoimmunity and arthralgia). FDR, first-degree relative; CSA, clinically suspect arthralgia; MSK, musculoskeletal; UA, undifferentiated arthritis.

if the risk of RA was 40%, whereas 30% and 7% would be willing to take preventive medication if the risks of RA were 20% and 1%, respectively. Attribute analyses revealed that the odds of accepting preventive treatment were higher if treatment was associated with a ≥20% reduction in the risk of developing RA compared with treatment that only delayed RA development, and was also higher for treatment associated with a lower risk of serious adverse events (≤10%) compared with a higher risk (>10%). Interestingly, several factors showed no association with willingness to take preventive medication (that is, these factors did not seem to influence an individual's decision), including a delay in the onset of RA (instead of its prevention), a risk of mild adverse events, and the mode of administration of the medication (oral, injection or infusion)[28]. Although larger studies on this subject are needed, as well as studies of individuals considered at risk because of their symptoms rather than because they have a FDR with RA, these data highlight the important influence of patient perceptions on willingness to take preventive medication and the contributing factors that should be taken into account when designing preventive trials and translating findings into clinical practice. Studies in the field of oncology and cardiovascular diseases have shown that adherence to preventive medications is rather poor and hence the willingness of patients to take such medication is of utmost importance[29,30].

RA prevention in clinical practice

Disease prevention in different healthcare settings. Disease prevention includes a wide range of procedures and interventions, all aimed at reducing the risks and threats to patient health. Primary, secondary and tertiary prevention are different in nature[31] (FIG. 2). Primary prevention aims to prevent disease before it occurs and can be directed at either the whole population, individuals at high risk of disease as a result of a particular factors (for example, individuals with specific genetic risk factors or individuals that smoke) or individuals of a specific age or sex. Examples of primary prevention are the immunization of young children and the screening and treatment of hypertension in a high-risk population (for example, individuals predicted to be at high risk based on their age, BMI and/or ethnicity) to prevent future cardiovascular events. Screening for the presence of certain serological factors (for example, RA-related autoantibodies) in the general population or in the FDRs of patients with RA, who have a threefold to fourfold increased risk of developing RA, can be considered to be relevant to primary intervention. Despite the increased risk of disease development in family members of patients with RA, the absolute risk in such individuals is low, as is the absolute risk of an asymptomatic individuals in the general population developing disease [32–34]. However, the features of primary prevention are outside the scope of this Review, and are not discussed further.

Secondary prevention aims to reduce the symptoms of a disease that has already occurred, such as joint pain. This process involves detecting and treating the disease as soon as possible to halt (or slow) disease progression. An example of secondary prevention is the regular screening of women over the age of 50 years for breast cancer by mammography. Although the phase in which RA starts is not completely clear, interventions performed in the symptomatic phase of arthralgia (the phase preceding clinical synovitis) can be considered a form of secondary prevention (FIG. 2). Tertiary prevention aims to mitigate the effects of an ongoing disease; in the case of RA, tertiary prevention concerns patients with clinical arthritis and/or RA, which is also beyond the scope of this Review.

In the context of RA, intervention aimed at secondary prevention begins with the identification of patients with arthralgia who might progress to RA. However, not all patients with arthralgia are similar, and the balance of whether or not to screen and/or treat a patient with arthralgia will depend on the pretest probability that a patient has an inflammatory form of arthralgia; this probability can vary depending on the health care setting (as discussed below).

Identifying patients at risk of developing RA. Patients at risk of developing RA can be identified by different approaches depending on the health care setting (FIG. 2). Screening for patients with arthralgia and secondary intervention can be performed in a primary (the general practice surgery) or secondary (the rheumatology outpatient clinic) health care setting. In primary care, interventions can be performed on all patients that present with any type of musculoskeletal symptoms. Although the exact numbers of individuals with musculoskeletal symptoms are unknown, such symptoms are a common complaint in primary care. However, for the vast majority of these patients, their symptoms will be unrelated to (imminent) RA and, although the exact numbers are unknown, the proportion of these patients that have suspected arthritis is probably small. In the United Kingdom, patients with RA have been reported to visit their general practitioner up to eight times before being referred to secondary care[35]; nonetheless, patients with (imminent) RA comprise a very small proportion of all patients visiting general practitioners[36].

Only some patients with any form of musculoskeletal symptoms are referred to secondary care, as these patients are generally only referred if the general practitioner judges that they have a high pretest probability of developing an inflammatory disease. Although referral criteria have been proposed for identifying patients with suspected early RA, such as the presence of metatarsophalangeal and/or metacarpophalangeal involvement and morning stiffness lasting ≥30 minutes[37], most general practitioners differentiate patients using their expertise. Although fewer patients with musculoskeletal symptoms visit secondary care than primary care, this population is still heterogeneous. Patients with either clinical arthritis or evident RA represent only a small proportion of those patients with musculoskeletal symptoms that are referred to secondary care[38]. Similarly, only a small proportion of these patients are considered to have clinically suspect arthritis (CSA; that is, patients with arthralgia without clinical arthritis but considered to be at risk of developing RA on the basis of their clinical presentation)[38]. A Dutch observational study showed that patients with CSA comprised only 6.5% of all patients that presented to rheumatologic care without clinical arthritis and with arthralgia that was otherwise unexplained[39]. In secondary care, pattern recognition and clinical expertise are important for differentiating patients with arthralgia who are at risk of developing RA from patients with other types of arthralgia.

In other words, not all patients with arthralgia are similar and the probability of a patient with arthralgia subsequently developing RA varies depending on the setting from which the patient is selected (FIG. 2). Patients with CSA, who have a higher probability of developing RA than a typical patient with arthralgia, constitute only a small subgroup of patients with arthralgia presenting in secondary care[37]. Importantly, a study in 2016 reported that clinical expertise (that is, the judgement that a patient has CSA) has a high sensitivity for identifying at-risk patients in secondary care (80%), and that few patients who present with arthralgia that later progress to RA are missed by their rheumatologists[38].

Although clinical expertise is regularly used in daily care, its subjectivity is an obvious drawback for scientific studies. Hence, a EULAR task force set out to explicate this particular clinical expertise in defined measurable terms and reached a definition for 'arthralgia suspicious for progression to RA' (REF. 40). This definition is intended to be used in secondary care in patients with arthralgia considered by the rheumatologist more likely to be imminent RA than other diagnoses (that is, patients with CSA). The clinical definition consists of seven items, five of which are obtained by history taking and two by physical examination (BOX 1). Health care systems around

Box 1 | **EULAR definition of arthralgia suspicious for progression to RA[38]**

A sensitive definition of arthralgia suspicious for progression to rheumatoid arthritis (RA) requires the presence of at least three of the seven items listed below*. A specific definition requires the presence of at least four of these items. This definition is designed to be used in patients with arthralgia without clinical arthritis and without another explanation for the arthralgia.

History taking

- Joint symptoms of recent onset (duration <1 year)
- Symptoms located in metacarpophalangeal joints
- Duration of morning stiffness ≥60 minutes
- Most severe symptoms present in the early morning
- Presence of a first-degree relative with RA

Physical examination

- Difficulty with making a fist
- Positive squeeze test of metacarpophalangeal joints

*The reported area under the curve (AUC) of this combination of parameters is 0.93. The sensitivity and specificity of this combination of parameters in the presence of three or more items are 90% and 74%, respectively. These values were calculated in a validation study with the clinical expertise of a group of European expert rheumatologist that evaluated patients in their own practices as reference[40]

the world are organized differently, with primary care being managed either by general practitioners or by specialists (such as internists, gynaecologists, orthopaedists or surgeons), resulting in different populations of patients with arthralgia. However, all these health care systems have rheumatologists who see patients with suspected imminent RA and, therefore, the EULAR definition of arthralgia suspicious for progression to RA is applicable in almost all health care systems. The aim of this definition is to harmonize what group of patients rheumatologists consider being at risk of developing RA. Indeed, data have revealed that this definition serves well to exclude some patients that (despite a rheumatologist's suspicion of imminent RA) actually have a low risk of RA. Additionally, the application of this definition in patients with CSA identified a subgroup of patients with a slightly higher risk of subsequent RA compared with the remaining patients with CSA[41].

In conclusion, selecting patients with arthralgia and a high risk of developing RA, such as patients who fulfil the EULAR definition of arthralgia suspicious for progression to RA, might offer an optimal starting position from which to investigate the mechanisms underlying this phase of RA development or to design preventive trials.

Predicting disease risk in different health care settings. Selecting the correct subgroup of individuals to test (risk stratification) is essential as this selection can influence the post-test probability of the tested population developing RA. This general principle is exemplified when considering ACPA status as a predictive indicator of RA development (TABLE 3). In the general population, the risk of ACPA-positive individuals developing RA over 5 years is estimated to be ~5%, with a lifetime risk of 16%[6,7]. The prevalence of ACPA-positive individuals in the general population is 1–2%[42–44], and the results from a longitudinal study in this setting suggest that the presence of ACPAs in symptom-free individuals is associated with an 8.5% risk of developing RA after ~3 years of follow-up[42]. These findings mean that 91.5% of ACPA-positive individuals will not develop RA in the forthcoming years (and hence these patients will have false-positive diagnoses when ACPA status is used as a measure for predicting RA development). Based on the prevalence of ACPA-positive individuals and the positive predictive value (PPV) of ACPA testing in the general population, the number of individuals in the general population that need be to tested in order to identify one patient who will develop RA can be estimated at ~1,200.

Several studies on ACPA-positive arthralgia have been performed in different settings (health fairs, primary care or secondary care, or combinations thereof)[45–47]. In these studies, the PPV of ACPA testing for RA development over 1 year ranged from 20% to 34%[45,46,48]. As the number of individuals that underwent ACPA testing was not reported, the number needed to test (NNT) in order to identify a patient that will progress onto developing RA cannot be estimated. 16% of patients with CSA are estimated to be ACPA-positive[11], and a positive ACPA test in such patients is associated with a 63% risk of developing clinical arthritis within 1 year; thus, in this subset of patients the risk of a false-positive test result, when using ACPA status as a predictor of arthritis development within 1 year, is 37%. Based on these data, the number of patients with CSA that need to be tested to identify one ACPA-positive patient who develops RA within 1 year is ten. Hence, the higher the *a priori* risk of developing RA, the higher the predictive value of ACPA testing for subsequent RA development (that is, the higher the PPV and the lower the risk of false-positivity) and thus the lower the NNT to identify one patient who will develop RA (TABLE 3). It is hoped that incorporating measurements of other structural features of ACPA, such as the presence of specific glycans in the Fab or Fc domain of ACPA molecules, will lead to better performance of ACPA assays[49,50].

Identifying imminent RA. Knowledge of ACPA status alone is insufficient to accurately stratify patients with arthralgia who are clinically at risk of developing RA (that is, patients with CSA), as the PPV of ACPA testing is at most 63%[11] (implying that ≥37% of ACPA-positive patients would have false-positive diagnoses), and up to half of the patients with newly diagnosed RA are ACPA-negative and hence are missed by this approach (false-negatives). Patients prefer tests that have a very high PPV (that is, a test that can confirm or exclude imminent RA). Hence, additional ways of stratifying patients are needed.

Table 3 | **Positive predictive value of ACPA testing for RA-development in different settings as observed in longitudinal studies**

Setting	Prevalence of ACPA-positive individuals	PPV of ACPA testing for RA development*	Estimated NNT to identify one patient with RA‡
General population	1–2%[42–44]	8.5% during a median of 3 years follow-up[42]	~1200
Patients with musculoskeletal symptoms	Unknown	Unknown	Unknown
Patients with CSA	16%[11]	63% within 1 year of follow-up[11]	10

For patients presenting with musculoskeletal symptoms in primary care, and unselected patients with musculoskeletal symptoms in secondary care, the prevalence of ACPA, the PPV of ACPA testing of such patients and the NNT to identify one patient who will develop RA is unknown. *Estimated PPV based on the number of ACPA-positive individuals who developed RA in the specified period. ‡Estimated NNT based on the prevalence and PPV; in the setting of the general population, the calculation was performed with a prevalence of 1%. ACPA, anti-citrullinated peptide antibody; CSA, clinically suspect arthralgia; NNT, number needed to test; PPV, positive predictive value; RA, rheumatoid arthritis.

Box 2 | **Research agenda for examining the prevention of progression from arthralgia to arthritis**

For the design and interpretation of preventive studies, and translating such findings into clinical practice, several questions remain to be addressed:

- Is it possible to predict with a high accuracy (for example, a positive predictive value of ≥80%) which patients with arthralgia will develop rheumatoid arthritis (RA), using symptoms, clinical signs and additional tests that are feasible to implement in clinical practice? And if so, how?
- Will any primary care tool(s) be able to identify patients with a high risk of developing arthritis and/or future RA, who should hence be referred to rheumatologic care? And if so, which ones?
- What biologic processes are responsible for the development of arthralgia and subclinical inflammation and which processes determine whether these features are progressive or will resolve spontaneously?
- What are the overlapping and non-overlapping pathways that contribute to the development of anti-citrullinated peptide antibody (ACPA)-positive and ACPA-negative RA?
- Can the development of clinically apparent persisting arthritis be prevented by treating patients in the symptomatic pre-arthritis phase (or does disease maturation occur at an earlier stage)?
- If proof-of-concept trials reveal beneficial effects of initiating treatment in the pre-arthritis phase, which drugs are most effective (and in which subset of patients)? And for how long should patients be treated to prevent RA development?
- What is an acceptable 'number needed to test' for tests that identify patients with RA in pre-arthritis stages?
- What is an acceptable 'number needed to treat' to prevent RA development?
- What personal and social factors determine a patient's willingness to start preventive treatment and adhere to such treatment?

Studies have identified other potential biomarkers for predicting RA progression. For example, subclinical joint inflammation, detected either by MRI or by ultrasonography, is a proven predictive indicator of RA development[9–11,51]. Further studies are required that directly compare the predictive accuracy of both imaging modalities, and that evaluate the minimal region needed to be imaged for maximal results; however, current data demonstrate that subclinical inflammation can predict RA development independently of autoantibody status and clinical features in patients with CSA, indicating that the presence of both autoantibodies and subclinical inflammation might further increase the risk of developing RA compared with the presence of each feature alone[10,11]. Increased levels of CRP can also independently predict RA development in such patients[11]. Finally, preliminary studies investigating the predictive value of certain B cell or T cell characteristics, as well as of gene expression profiles in whole blood, have shown promise[52–56]. Although these studies require replication, these markers are of interest as they might provide further insight into the aetiopathogenetic mechanisms of RA.

Several ongoing studies are investigating other predictors of RA development, such as autoantibodies other than ACPAs and structural features of autoantibodies; these studies include not only patients with arthralgia but also asymptomatic FDRs of patients with RA, in an attempt to look at individuals with a higher likelihood of developing RA than the general population[57–60]. Together these studies might provide additional information on RA development and help with the prediction of RA development in different at-risk populations.

Three separate studies have combined different types of predictors in patients with arthralgia to develop a prediction model. Unfortunately, these studies investigated different patient populations (ACPA-positive patients with nonspecific musculoskeletal symptoms in primary care, autoantibody-positive patients with arthralgia, and patients with CSA in secondary care) and so cannot be directly compared[11,45,46]. Although the results were promising, none of these models has yet been validated in independent patient populations. So, although information on different types of biomarkers are available, the use of different patient populations in these studies, in all of which the risk of developing RA is different, hampers the validation of each biomarker and/or model.

Several outstanding questions remain to be addressed when examining disease progression from arthralgia to arthritis (BOX 2). In order to be able to accurately predict RA development from the pre-arthritis phases, researchers should collaborate and use similar criteria (such as the EULAR definition of arthralgia suspicious for progression to RA) for evaluating clinically relevant patient groups. The harmonization of patient selection will enable researchers to combine the results of studies performed at different centres and to assess and/or validate findings from other centres. Furthermore, more extensive observational studies on the natural course of arthralgia in patients at risk of developing RA (without DMARD treatment) are needed to improve risk stratification. This research could reveal whether physicians should initiate preventive treatment and, if so, in which groups of patients.

Conclusions

The development of RA is a multistep process that can be ongoing for years before arthritis is present. Pre-arthritis phases might be part of the therapeutic window of opportunity and disease modulation during this phase is hypothesized to prevent clinically apparent and persistent RA from arising. To examine whether progression from arthralgia to arthritis can be prevented, correctly identifying patients (that is, accurate risk prediction) is crucial, and should overcome false-negative study results. Currently, several different approaches for

identifying at-risk populations are being tested and several trials are ongoing. However, whether disease modulation in the pre-arthritis phase has beneficial effects has not yet been demonstrated. Refining the term arthralgia and specifying the clinical characteristics of patients who have arthralgia and are at risk of developing RA, such as the EULAR definition of arthralgia at risk for RA, might reduce the heterogeneity of patients included in different studies. The EULAR definition is a sensitive predictor of RA development, and reflects expert opinion of imminent RA[41]. Therefore, this definition might offer an optimal starting position for investigating the mechanisms underlying this phase of RA development and designing preventive trials. Further research is needed to characterize the evolution from pre-arthritis to clinically overt disease in order to establish if disease modulation in this phase is effective in preventing RA (and if so, with which drugs).

1. Smolen, J. S. *et al.* EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann. Rheum. Dis.* **73**, 492–509 (2014).
2. Combe, B. *et al.* 2016 update of the EULAR recommendations for the management of early arthritis. *Ann. Rheum. Dis.* **76**, 948–959 (2017).
3. Boers, M. Understanding the window of opportunity concept in early rheumatoid arthritis. *Arthritis Rheum.* **48**, 1771–1774 (2003).
4. van Nies, J. A., Tsonaka, R., Gaujoux-Viala, C., Fautrel, B. & van der Helm-van Mil, A. H. M. Evaluating relationships between symptom duration and persistence of rheumatoid arthritis: does a window of opportunity exist? Results on the Leiden Early Arthritis Clinic and ESPOIR cohorts. *Ann. Rheum. Dis.* **74**, 806–812 (2015).
5. Ajeganova, S. *et al.* Disease-modifying antirheumatic drug-free sustained remission in rheumatoid arthritis: an increasingly achievable outcome with subsidence of disease symptoms. *Ann. Rheum. Dis.* **75**, 867–873 (2016).
6. Nielen, M. M. *et al.* Specific autoantibodies precede the symptoms of rheumatoid arthritis: A study of serial measurements in blood donors. *Arthritis Rheum.* **50**, 380–386 (2004).
7. Rantapää-Dahlqvist, S. *et al.* Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* **48**, 2741–2749 (2003).
8. Sokolove, J. *et al.* Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS ONE* **7**, e35296 (2012).
9. van de Stadt, L. A. *et al.* The value of ultrasonography in predicting arthritis in auto-antibody positive arthralgia patients: a prospective cohort study. *Arthritis Res. Ther.* **12**, R98 (2010).
10. Nam, J. L. *et al.* Ultrasound findings predict progression to inflammatory arthritis in anti-CCP antibody-positive patients without clinical synovitis. *Ann. Rheum. Dis.* **75**, 2060–2067 (2016).
11. van Steenbergen, H. W., Mangnus, L., Reijnierse, M., Huizinga, T. W. J. & van der Helm-van Mil, A. H. M. Clinical factors, anticitrullinated peptide antibodies and MRI-detected subclinical inflammation in relation to progression from clinically suspect arthralgia to arthritis. *Ann. Rheum. Dis.* **75**, 1824–1830 (2016).
12. van Steenbergen, H. W., Huizinga, T. W. & van der Helm-van Mil, A. H. The preclinical phase of rheumatoid arthritis: what is acknowledged and what needs to be assessed? *Arthritis Rheum.* **65**, 2219–2232 (2013).
13. Gerlag, D. M. *et al.* EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann. Rheum. Dis.* **71**, 638–641 (2012).
14. van Nies, J. A. *et al.* What is the evidence for the presence of a therapeutic window of opportunity in rheumatoid arthritis? A systematic literature review. *Ann. Rheum. Dis.* **73**, 861–870 (2014).
15. Dekkers, J. S., Schoones, J. W., Huizinga, T. W., Toes, R. E. & van der Helm-van Mil, A. H. Possibilities for preventive treatment in rheumatoid arthritis? Lessons from experimental animal models of arthritis: a systematic literature review and meta-analysis. *Ann. Rheum. Dis.* **76**, 458–467 (2017).
16. Bos, W. H., Dijkmans, B. A., Boers, M., van de Stadt, R. J. & Schaardenburg, D. Effect of dexamethasone on autoantibody levels and arthritis development in patients with arthralgia: a randomised trial. *Ann. Rheum. Dis.* **69**, 571–574 (2010).
17. Gerlag, D. M. *et al.* A single infusion of rituximab delays the onset of arthritis in subjects at high risk of developing RA [abstract]. *Arthritis Rheumatol.* **68**, (Suppl. 10), 3028 (2016).
18. van Dongen, H. *et al.* Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. *Arthritis Care Res.* **56**, 1424–1432.
19. Burgers, L. E., Allaart, C. F., Huizinga, T. W. J. & van der Helm-van Mil, A. H. M. Brief Report: Clinical trials aiming to prevent rheumatoid arthritis cannot detect prevention without adequate risk stratification: a trial of methotrexate versus placebo in undifferentiated arthritis as an example. *Arthritis Rheumatol.* **69**, 926–931 (2017).
20. Harrison, B. J., Symmons, D. P., Brennan, P., Barrett, E. M. & Silman, A. J. Natural remission in inflammatory polyarthritis: issues of definition and prediction. *Rheumatology* **35**, 1096–1100 (1996).
21. van Aken, J. *et al.* Comparison of long term outcome of patients with rheumatoid arthritis presenting with undifferentiated arthritis or with rheumatoid arthritis: an observational cohort study. *Ann. Rheum. Dis.* **65**, 20–25 (2006).
22. van der Helm-van Mil, A. H. M. *et al.* A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum.* **56**, 433–440 (2007).
23. van der Helm-van Mil, A. H. M. *et al.* Validation of a prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: moving toward individualized treatment decision-making. *Arthritis Rheum.* **58**, 2241–2247 (2008).
24. Aletaha, D. *et al.* 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann. Rheum. Dis.* **69**, 1580–1588 (2010).
25. Newsum, E. C., van der Helm-van Mil, A. H. & Kaptein, A. A. Views on clinically suspect arthralgia: a focus group study. *Clin. Rheumatol.* **35**, 1347–1352 (2016).
26. Stack, R. J. *et al.* Perceptions of risk and predictive testing held by the first-degree relatives of patients with rheumatoid arthritis in England, Austria and Germany: a qualitative study. *BMJ Open* **6**, e010555 (2016).
27. Sparks, J. A. *et al.* Randomized controlled trial of rheumatoid arthritis risk disclosure personalized to genetics, autoantibodies, and lifestyle among unaffected first-degree relatives: the Personalized Risk Estimator for RA (PRE-RA) family study [abstract]. *Arthritis Rheumatol.* **68** (Suppl. 10), 989 (2016).
28. Finckh, A., Escher, M., Liang, M. H. & Bansback, N. Preventive treatments for rheumatoid arthritis: issues regarding patient preferences. *Curr. Rheumatol. Rep.* **18**, 51 (2016).
29. Smith, S. G. *et al.* Factors affecting uptake and adherence to breast cancer chemoprevention: a systematic review and meta-analysis. *Ann. Oncol.* **27**, 575–590 (2016).
30. Naderi, S. H., Bestwick, J. P. & Wald, D. S. Adherence to drugs that prevent cardiovascular disease: meta-analysis on 376,162 patients. *Am. J. Med.* **125**, 882–887.e1 (2012).
31. Bonita, R., Beaglehole, R. & Kjellström, T. *Basic Epidemiology* 2nd edn (World Health Organization, 2006).
32. Frisell, T. *et al.* Familial risks and heritability of rheumatoid arthritis: role of rheumatoid factor/anti-citrullinated protein antibody status, number and type of affected relatives, sex, and age. *Arthritis Rheum.* **65**, 2773–2782 (2013).
33. Hemminki, K., Li, X., Sundquist, J. & Sundquist, K. Familial associations of rheumatoid arthritis with autoimmune diseases and related conditions. *Arthritis Rheum.* **60**, 661–668 (2009).
34. Scott, D. L., Wolfe, F. & Huizinga, T. W. Rheumatoid arthritis. *Lancet* **376**, 1094–1108 (2010).
35. National Audit Office. Services for people with rheumatoid arthritis. *National Audit Office* http://www.nao.org.uk/report/services-for-people-with-rheumatoid-arthritis/ (2009).
36. van den Dungen, C. *et al.* What factors explain the differences in morbidity estimations among general practice registration networks in the Netherlands? A first analysis. *Eur. J. Gen. Pract.* **14** (Suppl. 1), 53–62 (2008).
37. Emery, P. *et al.* Early referral recommendation for newly diagnosed rheumatoid arthritis: evidence based development of a clinical guide. *Ann. Rheum. Dis.* **61**, 290–297 (2002).
38. van Steenbergen, H. W. & van der Helm-van Mil, A. H. Clinical expertise and its accuracy in differentiating arthralgia patients at risk for rheumatoid arthritis from other patients presenting with joint symptoms. *Rheumatology* **55**, 1140–1141 (2016).
39. van Steenbergen, H. W. *et al.* Characterising arthralgia in the preclinical phase of rheumatoid arthritis using MRI. *Ann. Rheum. Dis.* **74**, 1225–1232 (2015).
40. van Steenbergen, H. W. *et al.* EULAR definition of arthralgia suspicious for progression to rheumatoid arthritis. *Ann. Rheum. Dis.* **76**, 491–496 (2017)
41. Burgers, L. *et al.* OP0073 Performance of the eular definition of arthralgia suspicious for progression to rheumatoid arthritis — a longitudinal study [abstract]. *Ann. Rheum. Dis.* **76 (Suppl. 2)**, 82 (2017).
42. Hensvold, A. H. *et al.* How well do ACPA discriminate and predict RA in the general population: a study based on 12 590 population-representative Swedish twins. *Ann. Rheum. Dis.* **76**, 119–125 (2017).
43. van Zanten, A. *et al.* Presence of anticitrullinated protein antibodies in a large population-based cohort from the Netherlands. *Ann. Rheum. Dis.* **76**, 1184–1190 (2017).
44. Haji, Y., Rokutanda, R., Kishimoto, M. & Okada, M. Anti-cyclic citrullinated protein antibody (ACPA) positivity in general population and follow-up results for ACPA positive persons [abstract]. *Arthritis Rheumatol.* **68** (Suppl. 10), 512 (2016).
45. van de Stadt, L. A., Witte, B. I., Bos, W. H. & van Schaardenburg, D. A prediction rule for the development of arthritis in seropositive arthralgia patients. *Ann. Rheum. Dis.* **72**, 1920–1926 (2013).
46. Rakieh, C. *et al.* Predicting the development of clinical arthritis in anti-CCP positive individuals with non-specific musculoskeletal symptoms: a prospective observational cohort study. *Ann. Rheum. Dis.* **74**, 1659–1666 (2015).
47. de Hair, M. J. *et al.* Smoking and overweight determine the likelihood of developing rheumatoid arthritis. *Ann. Rheum. Dis.* **72**, 1654–1658 (2013).
48. van de Stadt, L. A. *et al.* Development of the anti–citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. *Arthritis Rheum.* **63**, 3226–3233 (2011).
49. Rombouts, Y. *et al.* Anti-citrullinated protein antibodies acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of rheumatoid arthritis. *Ann. Rheum. Dis.* **74**, 234–241 (2015).

50. Rombouts, Y. *et al.* Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. *Ann. Rheum. Dis.* **75**, 578–585 (2016).
51. Kleyer, A. *et al.* High prevalence of tenosynovial inflammation before onset of rheumatoid arthritis and its link to progression to RA — a combined MRI/CT study. *Semin. Arthritis Rheum.* **46**, 143–150 (2016).
52. Janssen, K. M. J. *et al.* Regulatory CD4$^+$ T-cell subsets and anti-citrullinated protein antibody repertoire: potential biomarkers for arthritis development in seropositive arthralgia patients? *PLoS ONE* **11**, e0162101 (2016).
53. van Beers-Tas, M. H., Marotta, A., Boers, M., Maksymowych, W. P. & van Schaardenburg, D. A prospective cohort study of 14-3-3η in ACPA and/or RF-positive patients with arthralgia. *Arthritis Res. Ther.* **18**, 76 (2016).
54. Lübbers, J. *et al.* Changes in peripheral blood lymphocyte subsets during arthritis development in arthralgia patients. *Arthritis Res. Ther.* **18**, 205 (2016).
55. Chalan, P. *et al.* Circulating CD4$^+$CD161$^+$ T lymphocytes are increased in seropositive arthralgia patients but decreased in patients with newly diagnosed rheumatoid arthritis. *PLoS ONE* **8**, e79370 (2013).
56. van Baarsen, L. G. *et al.* Gene expression profiling in autoantibody-positive patients with arthralgia predicts development of arthritis. *Arthritis Rheum.* **62**, 694–704 (2010).
57. Kolfenbach, J. R. *et al.* A prospective approach to investigating the natural history of preclinical rheumatoid arthritis (RA) using first-degree relatives of probands with RA. *Arthritis Care Res.* **61**, 1735–1742 (2009).
58. National Institute for Health Research, Manchester Musculoskeletal Biomedical Research Unit. Pre-clinical evaluation of novel targets in RA. *PREVeNT RA* http://www.preventra.net/ (2017).
59. FNSNF. Arthritis-Checkup: Study of an early detection of the disease. *Arthritis-Checkup* www.arthritis-checkup.ch/index_gb.html (2017).
60. El-Gabalawy, H. S. *et al.* Immunogenetic risks of anti-cyclical citrullinated peptide antibodies in a North American native population with rheumatoid arthritis and their first-degree relatives. *J. Rheumatol.* **36**, 1130–1135 (2009).
61. ISRCTN registry. ISRCTN46017566 Arthritis prevention in the pre-clinical phase of rheumatoid arthritis with abatacept. *BioMed Central* http://dx.doi.org/10.1186/ISRCTN46017566 (2015).
62. US National Library of Medicine. *ClinicalTrials.gov* http://clinicaltrials.gov/ct2/show/NCT02778906 (2016).
63. Nederlands Trial Register. *Trialregister.nl* http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4853 (2014).
64. Nederlands Trial Register. *Trialregister.nl* http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=5265 (2015).
65. US National Library of Medicine. *ClinicalTrials.gov* http://clinicaltrials.gov/ct2/show/NCT02603146 (2017).

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Joint diseases: from connexins to gap junctions

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Abstract | Connexons form the basis of hemichannels and gap junctions. They are composed of six tetraspan proteins called connexins. Connexons can function as individual hemichannels, releasing cytosolic factors (such as ATP) into the pericellular environment. Alternatively, two hemichannel connexons from neighbouring cells can come together to form gap junctions, membrane-spanning channels that facilitate cell–cell communication by enabling signalling molecules of approximately 1 kDa to pass from one cell to an adjacent cell. Connexins are expressed in joint tissues including bone, cartilage, skeletal muscle and the synovium. Indicative of their importance as gap junction components, connexins are also known as gap junction proteins, but individual connexin proteins are gaining recognition for their channel-independent roles, which include scaffolding and signalling functions. Considerable evidence indicates that connexons contribute to the function of bone and muscle, but less is known about the function of connexons in other joint tissues. However, the implication that connexins and gap junctional channels might be involved in joint disease, including age-related bone loss, osteoarthritis and rheumatoid arthritis, emphasizes the need for further research into these areas and highlights the therapeutic potential of connexins.

Connexons are generated by the oligomerization of six tetramembrane-spanning connexin proteins (FIG. 1a,b). The resulting structures can function as hemichannels (FIG. 1c), mediating the release of small signalling molecules such as calcium and other ions, cyclic nucleotides, inositol phosphates, ATP and prostaglandins from inside the cell into the pericellular space[1,2]. Alternatively, connexon hemichannels from adjacent cells can also come together to form gap junctions (FIG. 1d), thereby facilitating the direct passage of signalling molecules of approximately 1 kDa (calcium and other ions, cyclic nucleotides, inositol phosphates, ATP and prostaglandins[3,4]) between neighbouring cells in a process known as gap-junctional intercellular communication (GJIC; FIG. 1e).

At least 21 connexin genes have been identified in humans[5], and 20 orthologous connexins in mice; a high degree of conservation exists between species. Each connexin is named after its predicted molecular weight, such that Cx43 (previously known as gap junction α1 protein), for example, has a predicted molecular weight of 43 kDa[6,7]. Structurally, connexins have cytosolic amino-terminal and carboxy-terminal ends, four membrane-spanning domains (M1, M2, M3 and M4), two extracellular loop domains (E1 and E2) and one cytosolic loop domain (FIG. 1b). The carboxyl terminus of a connexin reportedly functions as a scaffold, interacting with, and integrating signalling from, protein kinase C, mitogen-activated protein kinase, β-catenin, integrins, proto-oncogene tyrosine-protein kinase Src and tight junction protein ZO-1 (REFS 8–11). These interactions might alter the function of the binding partner; for instance, the binding of Cx43 to β-catenin might decrease the nuclear translocation of β-catenin. However, although connexins can exist as monomers, it is unclear whether they bind to other molecules as monomers or multimers.

Gap junctions and individual hemichannels are both gated and selectively permeable. That is, like other membrane channels, they open and close in response to stimuli, including changes in extracellular calcium concentrations and cytosolic pH, and only allow the passage of certain molecules[3,12]. For example, a channel composed of Cx43 is more permeable to anions than cations and is relatively large, permitting the diffusion of molecules ≤1 kDa, whereas a channel composed of Cx45 is small, has restricted permeability to molecules <0.3 kDa and primarily conducts cations. Cells often express more than one connexin, so connexons can be homomeric (formed from six monomers of the same connexin) or heteromeric (made up of different connexins) (FIG. 1a). Furthermore, hemichannels from opposing cells can engage in homotypic or heterotypic coupling[12,13] (FIG. 1d,e). In heteromeric connexons (FIG. 1a), the characteristics of

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Key points

- Multiple connexins are expressed in musculoskeletal tissues, including in joints
- Gap-junctional intercellular communication contributes to interconnected cell syncytium, which connect various cell types within joints
- Connexin dysfunction might contribute to joint disease
- Emerging data suggest that connexins might be novel targets for treating joint disease

one connexin typically predominate; thus, channels composed of Cx43 and Cx45 will have the permeability and gating characteristics of Cx45 (REF. 14).

Connexins are expressed throughout the musculoskeletal system, including in the bone, cartilage, skeletal muscle and the synovium, with Cx43 being the most widely expressed connexin in these tissues. Connexons are thought to carry out a fundamental role in organogenesis and homeostasis, and evidence suggests that connexon dysfunction might contribute to joint diseases such as skeletal muscle atrophy, rheumatoid arthritis (RA), osteoarthritis (OA) and osteoporosis. In this Review, we outline the structure and function of connexons, as well as the role of hemichannels and gap junctions in the development and function of joint tissues including bone, cartilage, synovium and muscle. We focus especially on the role of gap junctions and hemichannels in the adaptation of bone to mechanical load. We also discuss the role of dysfunctional connexons in joint disease and the potential for connexins to be novel therapeutic targets in joint diseases. Owing to the increased acceptance over the past few years that hemichannels can function independently of gap junctions, it remains to be investigated whether a phenotype that results from inhibition or deletion of a connexin is caused by a loss of hemichannel function, loss of GJIC, or both, or whether connexins might even carry out non-canonical, non-channel functions.

Connexons in bone

Connexin isoforms in bone. Bone is constantly being remodelled to fulfil its metabolic and mechanical functions. Osteoblasts are responsible for forming bone, whereas osteoclasts resorb or break down bone. Osteocytes, the most abundant type of cell in bone, are terminally differentiated osteoblastic cells that are embedded within a mineralized matrix. Osteocytes communicate with, and coordinate the activity of, osteoblasts and osteoclasts[15] in a manner that is highly dependent on GJIC. Cx43 is the predominant connexin in bone, and its expression by osteoblasts, osteocytes and osteoclasts[3], as well as by mesenchymal progenitor cells[16,17], enables communication between these cell types through gap junctions. Osteoblasts also express Cx37, Cx45 and Cx46 (REFS 18–20), although Cx46 in this cell type is largely confined to a monomeric form in the Golgi apparatus and does not contribute to GJIC[21]. Cx37 is also expressed in osteocytes and osteoclasts[22,23]. Thus, the bone cell network is a large interconnected syncytium (FIG. 2) that functions in coordinating bone function. This coordination might result from the function of connexins in gap junctions or hemichannels, or could be mediated by non-channel functions of connexins.

The role of connexins in skeletal growth and development. Mice with a global deficiency in Cx43 (encoded by *Gja1*) die shortly after birth owing to heart defects[24]. During embryonic development, such mouse pups display reduced mineralization of the axial and appendicular skeleton and the cranial vault, suggesting that Cx43 is critical for normal skeletal development as well as for heart development[25]. Interestingly, mice deficient in Cx43 solely in osteoblasts and osteocytes display no skeletal abnormalities at birth[26]. Mechanistically, Cx43 might promote skeletal development by increasing the activity of Runt-related transcription factor 2 (RUNX2; encoded by *Cbfa1*)[27], a master transcriptional regulator of osteogenesis[28]. Double heterozygous $Gja1^{+/-}Cbfa1^{+/-}$ mice have a skeletal phenotype that includes a marked increase in bone cross-sectional area and porosity, which is not observed in either $Gja1^{-/-}$ or $Cbfa1^{-/-}$ mice[29]. The importance of Cx43 in normal skeletal development is emphasized by the phenotype of individuals with oculodentodigital dysplasia. These patients have several mutations in *GJA1* and present with craniofacial abnormalities (such as skull hyperostosis, pointed nose and enamel hypoplasia), aplastic or hypoplastic middle phalanges, syndactyly and broad tubular long bones[11,30–32]. Taken together, these results indicate a necessary role for Cx43 in normal skeletal development.

Bone cell differentiation. Early *in vitro* studies showed that the expression of Cx43 parallels osteoblastic differentiation[33–35], and that inhibiting Cx43 expression reduced osteoblastic differentiation[16]. However, $Gja1^{-/-}$ mice display increased periosteal bone formation *in vivo*[36–38]. These seemingly contradictory results indicate that Cx43 expression and function in the skeleton is more complex than the original studies suggested. Examination of Cre-lox recombination models that facilitate the deletion of *Gja1* expression during various developmental stages revealed that the requirement for Cx43 in osteoblastic differentiation is dependent on the developmental stage. Cx43 deficiency early in the osteoblastic lineage led to interruption of osteoblastic differentiation and mineralization, whereas Cx43 deficiency later in the osteoblastic lineage did not dramatically affect osteoblast differentiation[25,36,38]. Whether Cx43 is fundamentally necessary for osteoclast differentiation remains unclear. *In vitro*, Cx43-mediated GJIC is critical for osteoclast differentiation and pre-osteoclast fusion[39–41], but studies *in vivo* have yet to demonstrate a direct role for Cx43 in osteoclastogenesis.

Mechanotransduction. The functional coupling of gap junctions between osteocytes and osteoblasts[42,43] and the mechanosensory capacity of these cells[44] has led to the suggestion that GJIC might contribute to mechanotransduction[45]. In support of this notion, mechanical signals regulate the levels of Cx43 and GJIC between bone cells[46–51], GJIC sensitizes bone cell networks to diverse extracellular signals[26,52,53], and mechanically induced signals are communicated among bone cells via gap junctions[43,54,55]. These data have led to a near-consensus among researchers in the field that GJIC, hemichannels or connexins facilitate the anabolic response of bone to

Cre-lox recombination
A site-specific recombinase technology that is used to produce deletions, insertions, translocations and inversions at specific sites in the DNA of cells.

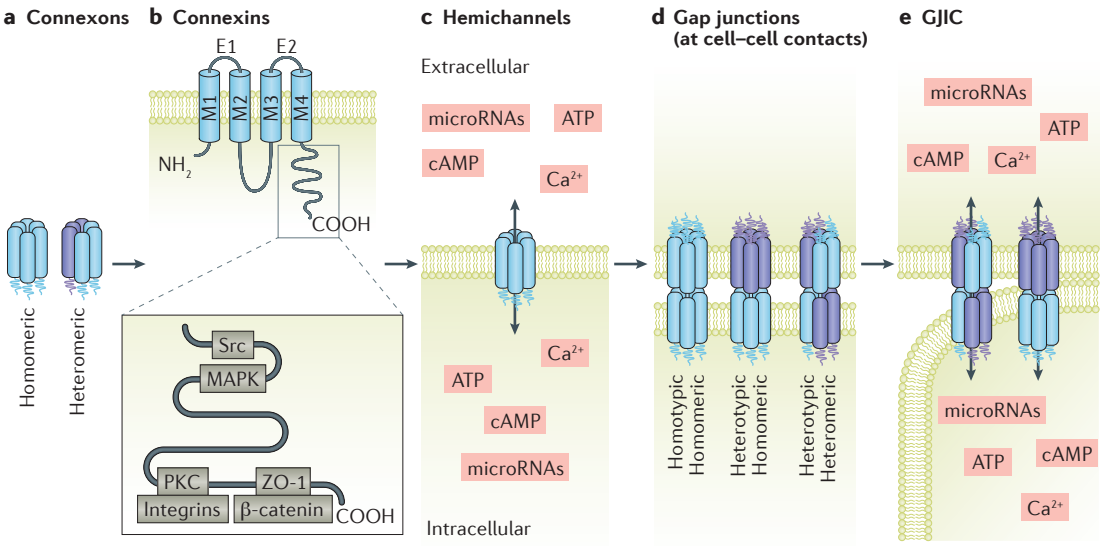

Figure 1 | **Connexins, connexons, hemichannels and gap junctions. a** | Connexons are formed from the oligomerization of six connexins; connexons can be homomeric (formed from six monomers of the same connexin) or heteromeric (made up of different connexins). **b** | Each connexin has four transmembrane domains (M1, M2, M3 and M4) and two extracellular loops (E1 and E2); the intracellular carboxyl terminus of a connexin can interact with proto-oncogene tyrosine-protein kinase Src, mitogen-activated protein kinase (MAPK), protein kinase C (PKC), β-catenin and integrins. **c** | Connexon hemichannels facilitate the flux of small molecules such as cyclic AMP (cAMP), ATP, microRNAs and calcium ions across the plasma membrane. **d** | Hemichannels from adjacent cells form gap junctions by engaging in homotypic or heterotypic coupling. **e** | Varied gap junction composition mediates permeability, selectivity and gating in gap-junctional intercellular communication (GJIC). ZO-1, tight junction protein ZO-1.

mechanical load[45,56–58]; however, *in vivo* evidence suggests the opposite is true. As with investigations of osteoblast differentiation, the point at which *Gja1* is deleted determines the skeletal responsiveness to loads. When *Gja1* is deleted in osteoblasts (*2.3 kb–Col1a1–cre; Gja1*$^{flox/flox}$ mice), the endosteal bone formation rate is similar between wild-type and knockout mice under normal loading conditions, but is attenuated in knockout mice in response to three-point bending[59]. Using a Cre–*lox* system to delete *Gja1* in mature osteoblasts (*Bglap*–cre; *Gja1*$^{flox/flox}$ mice), the periosteal bone formation rate increased in knockout mice relative to wild-type controls, and there was a greater increase in bone formation rate in response to cantilever loading in Cx43-deficient mice[60]. Similar results were observed in response to anabolic loading when *Gja1* was deleted in mesodermal progenitor cells[38] or in osteocytes[61]. Cx43 also mediates the skeletal response to unloading. *Bglap*–cre; *Gja1*$^{flox/flox}$ mice are less sensitive to hindlimb suspension-induced bone loss than wild-type controls[62]. Similar results were observed in muscle-paralysis-induced bone loss in *2.3 kb–Col1a1–cre; Gja1*$^{flox/flox}$ mice[63]. Thus, in the absence of Cx43, bone is more responsive to the anabolic effect of mechanical load and less responsive to the catabolic effects of unloading.

The mechanism underlying the role of Cx43 in the response of bone to its mechanical environment is unknown. However, emerging evidence points to the involvement of the sclerostin–Wnt–β-catenin pathway, which is already strongly implicated in the mechanism underlying the responsiveness of bone to mechanical load[62,64,65], and the potential regulation of this process by Cx43 (REFS 37,38,62). Furthermore, regulation of receptor activator of nuclear factor-κB ligand (RANKL), a pro-osteoclastogenic factor, and osteoprotegerin, an anti-osteoclastogenic factor, occurs in response to mechanical load, perhaps through Cx43 (REFS 37,51,60). *Gja1* is also a target gene for canonical signalling by β-catenin[66], the transcription of which increases in response to anabolic loading[67]. These data suggest a potential positive feedback loop, wherein loading increases Wnt–β-catenin signalling, which drives *Gja1* transcription, which then further sensitizes osteoblasts to increased load. In summary, the role of Cx43 in bone responsiveness to its mechanical environment might involve well-characterized signalling pathways that are already known to regulate osteoblast and osteoclast behaviour. Specific roles for hemichannels versus gap junctions in skeletal mechanotransduction were demonstrated by generating mice that were incapable of GJIC but retained the capacity for hemichannel function. Osteocytic Cx43 channels contributed to the skeleton both as hemichannels and gap junctions, with hemichannels primarily responsible for guiding osteocyte survival, endocortical bone resorption and periosteal bone apposition and with GJIC mediating remodelling[68]. However, more research is required to understand exactly how Cx43, through hemichannels and gap junctions, contributes to bone adaptation to mechanical load.

Anabolic loading
Mechanical loading that increases the abundance of bone.

Connexons in cartilage

Chondrocytes are responsible for the development and maintenance of cartilage and generate a unique ultrastructure in terms of biochemical composition and

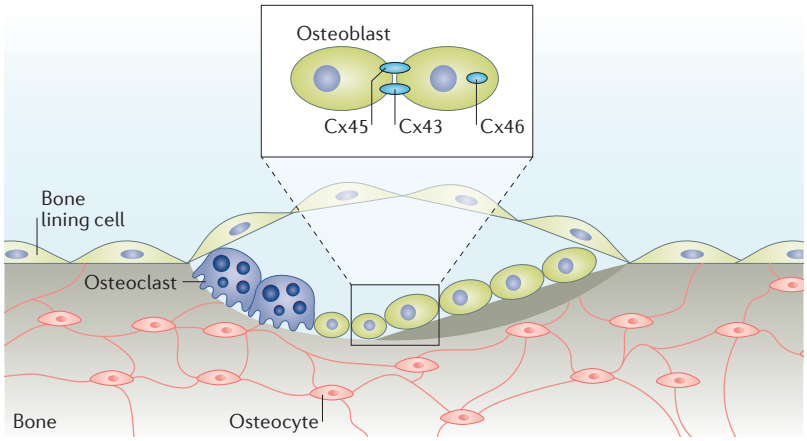

Figure 2 | **Connexins in bone.** Gap-junctional intercellular communication (GJIC) has been demonstrated between bone lining cells, osteoblasts, osteocytes and osteoclasts. Cx43 is the predominant connexin in bone, but Cx45 also contributes to GJIC. Cx46 is expressed by osteoblasts but largely remains in the cytosol.

biophysical properties[69]. By varying the composition of this matrix (variables include, for example, the type of collagen, amount of non-collagenous protein, water content and glycosaminoglycan properties), chondrocytes produce three types of cartilage: fibrocartilage, elastic cartilage and hyaline cartilage. Each type of cartilage lubricates and resists tension, compression, bending or shear. To date, neither gap junctions nor their component connexins have been identified in elastic cartilage. However, connexin isoforms have been documented in fibrocartilage and in hyaline cartilage.

Fibrocartilage. Fibrocartilage is associated with dense connective tissue and functions primarily to resist deformation under stress. Fibrocartilage is found in intervertebral discs, annulus fibrosis, pubic symphysis, menisci, the temporomandibular joint and entheses. Cx29 is expressed in the fibrocartilage of intervertebral discs and vertebral epiphyses[70]. Both Cx32 and Cx43 are expressed in rat Achilles tendon, although the expression of both connexins decreases as the tendon blends into uncalcified fibrocartilage[71]. Cx32 and Cx43 are also absent from calcified fibrocartilage, which indicates that GJIC (and hemichannel function) is absent between the tendon and bone compartments[71]. However, other studies reveal the presence of connexins in fibrocartilage at different anatomic locations. Cx43 was evident by confocal microscopy in every region of the annulus fibrosus of the intervertebral disc, being found along processes extending from the cell bodies, along the membrane of cell bodies and within cell bodies. Cx43 was also found at the tip of cytoplasmic processes even in cells that lacked close neighbouring cells[72], implying that Cx43 in this location is associated with hemichannels rather than with gap junctions. In meniscal cells within fibrocartilage, Cx43 immunostaining revealed a punctate expression on the plasmalemma, suggesting that functional GJIC occurs or that hemichannels function at this location. Cx43 expression was not observed in the hyaline cartilage-like inner third of the meniscus[73]. Cx45 expression has also been observed in the annulus fibrosus and expression of both Cx43 and Cx45 decreased with increasing age[74]. Although current evidence demonstrates that Cx29, Cx43 and Cx45 are expressed in fibrocartilage, studies to date have not yet determined whether these proteins form gap junctions or hemichannels, or have a channel-independent function, nor have they examined the consequence of inhibiting hemichannels or GJIC on fibrocartilage development and function. Thus, the exact role of connexins in fibrocartilage is unknown.

Hyaline cartilage. Hyaline cartilage forms the architectural basis for articular cartilage and the growth plate, which are crucial for locomotion and postnatal growth, respectively. Chondrocytes in hyaline cartilage express a variety of connexin isoforms, including Cx43 and Cx45 (REFS 75,76); the fact that chondrocytes are generally isolated within lacunae would suggest that these connexins do not participate in GJIC in this context, but connexins could function in hemichannels or have other gap junction-independent functions (FIG. 3). Cx32, Cx43, Cx45 and Cx46 are all present in the superficial, middle and deep zones of growth plate cartilage[76]. Cx45 is diffusely expressed throughout the chondrocytic cytosol and forms small spots around the margin of cells[76]. Cx43 localizes to the margin of cells and is expressed in 80–100% of chondrocytes in each zone; it forms gap junctions when a lacuna contains multiple chondrocytes[76] (FIG. 3). However, contrasting results in separate studies in healthy human articular cartilage[75] and in rodents[77] revealed that Cx43 was expressed primarily in the superficial zone of the growth plate, with expression decreasing in the middle and deep zones of the growth plate. The reason for these contrasting results is currently unknown.

In vitro, articular cartilage chondrocytes express both Cx43 (REF. 78) and Cx45 (REF. 76) and demonstrate the hallmarks of GJIC, including fluorescent dye transfer[78], electrical coupling[76] and glucose and amino acid flux[79]. Cultured articular chondrocytes can also transduce mechanical signals from a stimulated cell to non-stimulated cells[78,80], and this signal spreading is inhibited by glycyrrhetinic acid, a GJIC blocker. Connexins might regulate chondrogenesis, as glycyrrhetinic acid prevents the chondrogenic differentiation of mesenchymal stem cells[81] and prechondrogenic cell condensation[82]. However, these studies should be interpreted with caution as glycyrrhetinic acid also blocks pannexin channels[83]. Furthermore, the relative isolation of chondrocytes *in vivo* is thought to preclude a function for connexons in GJIC; instead, connexons in chondrocytes are likely to function primarily as hemichannels, which are capable of releasing molecules of <1 kDa into the pericellular environment. Isolated bovine articular chondrocytes trapped within an agarose gel demonstrated hemichannel activity, showing an increased uptake of Lucifer yellow in the absence of extracellular calcium[75] or in response to mechanical loading[84]. Dye uptake was attenuated by the addition of the connexon channel inhibitor flufenamic acid, suggesting that connexon hemichannels mediated this response. However, these data did not reveal whether

Pannexin channels
A family of vertebrate proteins that predominantly exist as large transmembrane channels connecting the intracellular and extracellular space.

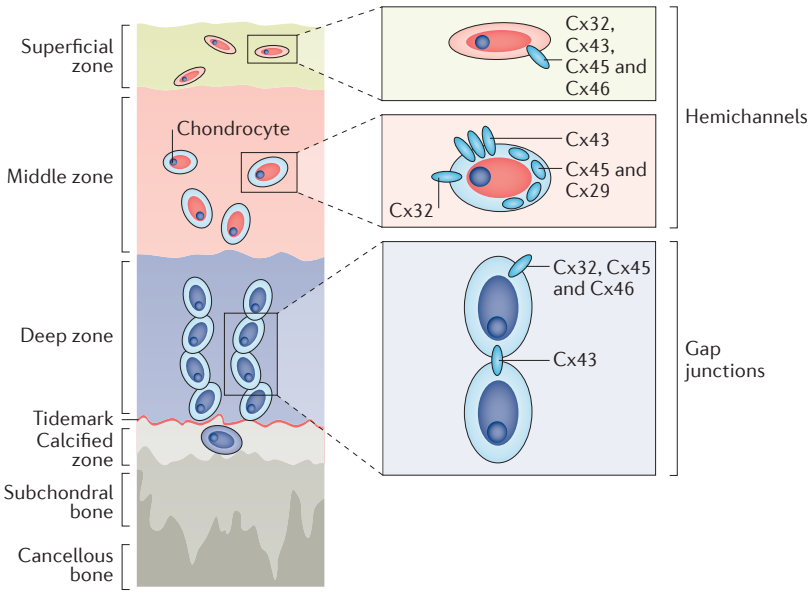

Figure 3 | **Connexins in cartilage.** Cx32, Cx43, Cx45 and Cx46 are expressed throughout the superficial, middle and deep zones of cartilage. Cx29 has been detected in the middle zone. As chondrocytes exist largely as isolated cells throughout the superficial and middle zones, connexons in these zones function as hemichannels rather than as gap junctions. Gap-junctional intercellular communication (GJIC) through Cx43 might occur in chondrocytes within the deep zone or in chondrons containing multiple chondrocytes.

hemichannel activity and dye uptake were mediated by Cx43 or Cx45, nor did they identify the molecules that are released from chondrocyte hemichannels. Evidence from other cell types suggests that ATP is released from hemichannels. For example, in cortical astrocytes[85,86], corneal endothelial cells[87] and osteocytes[1], hemichannel opening promotes the release of ATP. Once released, ATP binds to and activates ionotropic P2X and metabotropic P2Y purinergic receptors to mobilize calcium within a target cell and to activate calcium-sensitive signalling cascades[88]. ATP was released from chondrocytes in chondron pellets[89] and agarose-entrapped chondrocytes[84] that had been exposed to cyclic loading, or primary or clonal chondrocytes exposed to fluid shear stress[90], indicating that ATP release from chondrocyte hemichannels might be involved in cartilage adaptation to mechanical load.

Connexins in synovium

The synovium of diarthrodial joints (FIG. 4) comprises macrophage-like type A cells, which are responsible for phagocytosing synovial fluid, and fibroblast-like type B cells, which produce hyaluronic acid and mucin to nourish and mechanically protect, respectively, the underlying articular cartilage. Electron-dense granules present on apposing plasmalemma that are characteristic of the architecture of gap junctions were first observed in rabbit and cat synovia by Groth[91], and later in human synovium by Dryll *et al.*[92]. Gap junctions exist primarily between type A cells, and to a lesser extent between type A and type B cells, and between type B cells[93]. Cx43 is present in the synovium *in vivo*, and it is speculated that Cx26 and Cx32 are also expressed[93]. Fibroblast-like synoviocytes express Cx43[94] and can participate in GJIC with heterologous cells such as chondrocytes *in vitro*: during this process, GJIC and purinoreceptor activation mediate the propagation of calcium waves from mechanically stimulated chondrocytes to co-cultured synoviocytes, and *vice versa*[95]. However, as discussed above in the context of chondrocytes, little current evidence exists as to how and when connexins, hemichannels or gap junctions influence the development and homeostasis of the synovium. Some evidence, discussed in greater depth below, does indicate that altered Cx43 expression and GJIC have an obligate role in the development and progression of OA.

Connexins in muscle

Connexins are expressed in myocytes during myogenic development and are upregulated in response to injury[96], but differentiated myofibres do not express connexins. Cx39 is expressed in myogenin-positive myoblasts and young myotubes from embryonic day 11.5 to birth in mice and rats, after which expression declines[97,98], suggesting a role for Cx39 during myogenic differentiation[97–99]. Cx39 deletion had no effect on the phenotype of murine skeletal muscle in the resting state, yet increased the expression of myogenin, myoblast determination protein 1 (MyoD) and Cx43 both during development and in models of muscle injury[100]. However, Cx39 might not function as a gap junction channel or hemichannel in this context as exogenously expressed Cx39 failed to mediate the transfer of microinjected dyes or tracers in gap-junction-deficient HeLa cells[97]. Cx40 is expressed briefly during early differentiation and localizes to areas in which myoblasts fuse into multinucleated myotubes[101].

Compared to Cx39 and Cx40, much more is known about the expression and function of Cx43 and Cx45 in muscle development; both proteins are implicated in myogenic commitment and differentiation. The expression of Cx43 decreases after myoblast fusion into myotubes[102], and inhibiting GJIC prevents myogenic differentiation[103]. Similarly, inhibition of GJIC in skeletal muscle satellite cells results in decreased MyoD expression, reduced myotube formation and increased satellite cell adipocytic differentiation[104]. However, as many of these results were obtained before the acceptance that connexons can function as hemichannels, it was not considered whether connexons functioned as hemichannels or participated in GJIC.

Muscle injury and muscle diseases alter the expression of connexins. Innervation reduces the expression of Cx39, Cx43 and Cx45 in myoblasts, and these connexins are absent in fully differentiated myofibres[105]. Similarly, muscle satellite cells, which proliferate before fusion into myofibres, express Cx43 and Cx45, and the absence of Cx43 or Cx45 prevents myofibre repair[106]. By contrast, trauma or disease increases connexin expression, which potentiates skeletal muscle damage. Denervation of fast myofibres increases the expression of Cx43 and Cx45, the hemichannel functions of which are required for activation of the inflammasome and for muscle atrophy[107]. Similarly, fast skeletal myofibres from a murine model of skeletal Duchenne muscular dystrophy express

Chondron pellets
Groups of chondrocytes and their adjacent pericellular environment that have been centrifuged to form dense pellets.

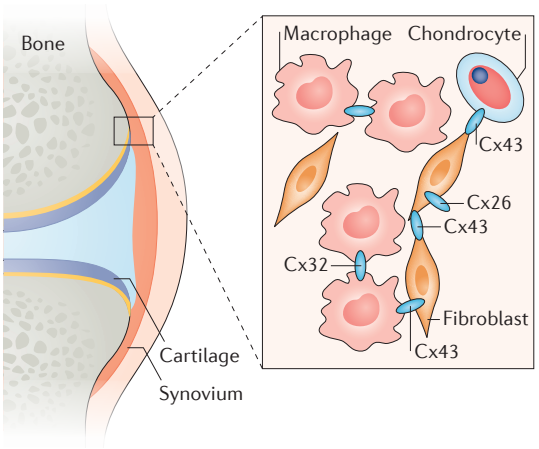

Figure 4 | **Connexins in synovium.** Cx43 has been detected in fibroblast-like synoviocytes and synovial macrophages, with evidence for gap-junctional intercellular communication (GJIC) between synovial macrophages, and between macrophages and fibroblast-like synioviocytes. Furthermore, GJIC between fibroblast-like synoviocytes and articular chondrocytes enables the propagation of calcium waves. Cx26 and Cx32 might also be expressed in synovial tissue.

Cx39, Cx43 and Cx45, which contribute to increased sarcolemmal permeability, also through hemichannel function[108]. Thus, in skeletal muscle, hemichannels have a causative role in tissue deterioration.

Connexins in musculoskeletal diseases

Osteoporosis. An age-related reduction in the expression of Cx43 has been observed in many tissues and cell types, including human dental pulp, endothelial cells and cardiomyocytes[109–111]; indeed, reduced Cx43 expression "appears to be a common feature of ageing cells" (REF. 112). Thus, it has been proposed that decreased Cx43 expression, GJIC or hemichannel activity could be a function of ageing that might lead to age-related osteopenia[113].

Osteoporosis is an important health problem that affects over 200 million people worldwide[114]. Even considering the effects and consequences of this disease, very little is known of its aetiology. The bone phenotype of Cx43-deficient mice, which includes periosteal and endosteal expansion, cortical thinning and increased porosity[11,60], indicates that changes in the expression of Cx43 might contribute to age-related bone loss. Parathyroid hormone (PTH) is known to have an anabolic effect on bone mass by inhibiting apoptosis in osteoblasts; PTH confers this anti-apoptotic effect by stimulating the accumulation of cyclic AMP (cAMP) in a manner that is dependent on Cx43[115]. Furthermore, PTH-stimulated increases in adenylyl cyclase activity and cAMP accumulation are attenuated in rat osteoblastic cells as a result of ageing[113]. Thus, the Cx43-dependent and cAMP-dependent anti-apoptotic effect of PTH might decrease as a function of age. This decrease, in turn, might contribute to age-related bone loss. Cx43 might also be involved in the mechanism by which bisphosphonates affect bone. Bisphosphonates, including alendronate, not only inhibit osteoclastic bone resorption but also prevent osteoblast and osteocyte apoptosis, and this anti-apoptotic effect is crucially dependent on the presence of sufficient levels of Cx43 in osteoblasts and osteocytes[116]. Thus, in addition to being involved in age-related bone loss, Cx43 is also important in the mechanism underlying the therapeutic efficacy of bisphosphonates.

Additional evidence for a role for connexins in osteoporosis comes from studies on fracture healing. A hallmark of osteoporosis is an increased fracture rate and delayed fracture healing, and previous studies have demonstrated that osteoblast differentiation and fracture repair are impaired in Cx43-deficient mice[117]. Thus, an age-related decrease in Cx43 expression or gap junction or hemichannel function might contribute to the delayed fracture healing that is typical of osteoporosis. These findings, together with results showing that Cx43 deficiency in osteoblasts and osteocytes enhances the anabolic effects of bone loading and protects against the catabolic effects of unloading[118], suggest that the role of Cx43 in bone is complicated and context-dependent, and emphasizes the need for future studies on the relationship between Cx43 and GJIC and age-related bone loss.

Osteoarthritis. OA is a common age-related degenerative joint disorder that causes pain, joint swelling and limited mobility. Historically, OA was considered to be a sequelae of ageing, resulting from focal 'wear and tear' on articular cartilage in response to mechanical damage incurred through life-long exercise[119]. However, the presence of synoviocyte hypertrophy, pro-inflammatory cytokines and differentially affected subchondral bone compartments demonstrates that OA is not a focal disease, but is instead a non-classical inflammatory disease of diarthrodial joints. Among the changes seen in the osteoarthritic joint are the production of the pro-inflammatory cytokines IL-1β and TNF, which in turn induce the expression of other cytokines (IL-6 and IL-8), chemokines (monocyte chemotactic protein 1 and granulocyte-macrophage colony-stimulating factor) and catabolic enzymes that are responsible for breakdown of cartilage and proteoglycans (matrix metalloproteinases (MMPs) and aggrecanases), ultimately leading to loss of tensile strength[120]. The current dogma is that both mechanics and genetics contribute to the development and progression of OA, depending on the anatomic location[121].

Connexins are expressed in joint tissues and, as connexons are implicated as homeostatic regulators in bone and muscle, it is tempting to speculate that joint tissue connexons might be involved in the pathogenesis of OA. Indeed, Cx43 has been detected in meniscal cell clusters, and it has been suggested that its presence might be related to the development of OA[73,122]. Genome-wide association studies have yet to identify polymorphisms in *GJA1* that influence susceptibility to OA, but polymorphisms in the gene encoding growth and differentiation factor 5 (GDF5) have been reported[123,124]. GDF5 increases the expression of *Gja1 in vitro*[125], and *Gdf5* and *Gja1* transcripts are both spatiotemporally coincident during organogenesis in mice[126]. These data suggest a link between *GDF5, GJA1* and OA.

Gap junctions and GJIC are altered, in terms of expression and function, throughout diarthrodial joints in osteoarthritic conditions. The levels of Cx43 and Cx45 (REF 76) are increased in cartilage tissue in patients with OA, as are the number of gap junction plaques[127]. Increased Cx43 expression is observed in chondrocytes within the damaged superficial zone and middle zone cartilage in patients with OA[76]; notably, staining for proliferative cell nuclear antigen, a marker of cellular proliferation, correlated with Cx43 staining in areas of damaged cartilage. However, the mechanistic function of Cx43 in OA, and whether it mediates progression of the disease or is simply increased as a consequence, remains unexamined. Whether increased expression of Cx43 mediates an increase in GJIC among articular chondrocytes has not yet been demonstrated but, because articular chondrocytes seem to exchange nutrients and amino acids through GJIC[79], it is tempting to speculate that the increased expression of Cx43 and/or Cx45 might be a compensatory mechanism to increase GJIC and to thereby facilitate nutrient exchange among articular chondrocytes.

Cx43 might also be involved in OA through channel-independent mechanisms. For instance, Cx43 can function as a scaffold protein[128]; its interactions with, and binding to, cytoskeletal proteins regulate cytoskeletal architecture[129] and cell proliferation[130,131]. Proteomic analysis of articular cartilage from healthy individuals and patients with OA investigated the variety and composition of Cx43-interacting proteins and identified differential interactions that occurred between health and disease conditions[132]. For example, in articular cartilage samples from healthy individuals, Cx43 immunoprecipitated with a number of proteins involved in aspects of cell metabolism such as glycolysis and gluconeogenesis (for example, aldolase A, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase 1, enolase 1, pyruvate kinase muscle isozyme 2, superoxide dismutase 2 and ATP synthase subunit F). Such interactions were not detected in samples from individuals with OA; instead, samples from these individuals revealed interactions of Cx43 with proteins involved in cytoskeletal organization and cell adhesion[132].

Under healthy conditions, synoviocytes produce synovial fluid to nourish and lubricate articular cartilage, thereby contributing to cartilage homeostasis. In OA, however, the synovium contributes to articular cartilage catabolism. By-products of the breakdown of cartilage extracellular matrix, such as fibronectin and collagen fragments, induce inflammation in extant chondrocytes[133,134] and the adjacent synovium[135]. As outlined above, activated macrophage-like synovial cells express TNF and IL-1β, which, in turn, induce fibroblast-like synoviocytes to secrete other chemokines and cytokines. Concomitantly, MMP-1, MMP-3, MMP-9 and MMP-13 are expressed by fibroblast-like synoviocytes and further contribute to cartilage degradation[136]. Similar to the situation in articular chondrocytes, the expression of Cx43 is increased in the synovium of patients with OA compared with that of healthy individuals[127], and is positively regulated by IL-1β in both fibroblast-like synoviocytes[137] and chondrocytes[138,139]. Overexpression of Cx43 in fibroblast-like synoviocytes increases the expression of MMPs, aggrecanases and pro-inflammatory cytokines through a mechanism that is dependent on nuclear factor-κB[140].

Rheumatoid arthritis. As expression of Cx43 is observed in all tissues of the joint, it has been suggested that Cx43 might be involved in RA in addition to its involvement in OA. RA is a joint disease that is characterized by greatly increased proliferation of synovial cells and eventual bone destruction[141]. As is the case with OA, pro-inflammatory cytokines are involved in RA. Increased GJIC contributes to cellular proliferation in many tissues and, as synoviocyte proliferation is increased in RA, increased GJIC mediated by Cx43 might also contribute to the development of this disease. Evidence supporting this concept comes from a study that demonstrated that levels of Cx43 mRNA are increased in fibroblast-like synoviocytes exposed to the inflammatory stimulus lipopolysaccharide (LPS), and also in the synovium of rats with collagen-induced arthritis[141]. Importantly, exposure to small interfering RNA targeted against Cx43 inhibited the LPS-induced expression of inflammatory cytokines in fibroblast-like synoviocytes and suppressed the progression of collagen-induced arthritis in rats. These studies, taken together with those described in the previous paragraph, suggest that Cx43 and GJIC might contribute to, and therefore be attractive therapeutic targets for, both RA and OA.

Conclusions

A plethora of *in vitro*, *ex vivo* and *in vivo* studies have documented the spatiotemporal expression of connexins during the development, homeostasis and repair of the musculoskeletal system. Furthermore, pharmacologic and genetic approaches have begun to elucidate the cellular and molecular functions of connexins in this system. Although the results of these studies imply that connexins contribute to tissue development and homeostasis, our understanding of when and how they influence these processes is incomplete. Emerging evidence suggests that connexins can function in their own right as scaffold and signalling proteins, as well as being key components of connexons in hemichannels and gap junctions. The development of approaches that can specifically target GJIC, hemichannel activity or non-channel connexin function is required to understand how each of these processes contributes to cell function.

As outlined above, the use of conditional mouse models to selectively delete *Gja1* in osteoblasts versus osteocytes, or to eliminate the capacity to undergo GJIC while retaining hemichannel function, has yielded unexpected results that have fundamentally altered our expectations of how connexons influence skeletal development and response to mechanical loads. However, fundamental knowledge of cellular and molecular signalling through connexins in cartilage, tendon, and muscle is practically non-existent. Mechanistic evaluation and causality of connexin function are inferred, but not demonstrated. For example, although connexins are present in tendons, whether they are required for tenogenesis during

development, or whether they have a role in repair, has not yet been functionally examined. Frequently, data are limited to a combination of *in vivo*, *ex vivo* and *in vitro* studies using global knockout animals. For example, our understanding of the role of Cx43 in myogenic commitment and differentiation would be greatly illuminated by studying the phenotype of mice with a conditional deletion of *Gja1* in muscle. The use of the Cre–*lox* system would further leverage such mouse models to evaluate the requirement of *Gja1* in muscle repair. Similarly, since the expression of *Gja1* increases in OA and is a predictor of disease severity, it would be interesting to know how the temporal deletion of *Gja1* in hypertrophic chondrocytes might alter OA progression.

Although much is known regarding the function of connexins in gap junctions in bone and muscle, relatively little is known regarding the function of gap junctions in other joint tissues. Thus, an appreciation of the role of gap junctions in joint disease is only just emerging. Strong evidence exists supporting a role for gap junctions in age-related bone loss and, possibly, in OA, but the relative lack of information regarding the function of connexins in the synovium, fibrocartilage and skeletal muscle precludes a complete understanding of the role of hemichannels and gap junctions in joint disease. Future studies focusing on the role of connexins in synovium, tendons and ligaments, particularly on how these tissues use gap junctions to integrate with bone and cartilage, will provide a better understanding of the role of gap junctions in joint disease and might uncover innovative therapeutic approaches. Currently, the major limitations to translating basic information about connexins into a therapy are fundamental gaps in our knowledge relating to connexin function in non-skeletal tissues, and the capacity to selectively activate constituent components of connexins and connexons. Likewise, as more information emerges regarding the potential of targeting connexins for treating joint disease, consideration must be given to potential off-target effects. Connexins are clearly important in the development and function of many tissue systems, including the cardiovascular system, central nervous system, skin and eyes; therefore, any therapeutic targeting of joints would have to be localized so as to avoid affecting other organs. Several examples that facilitate the localized delivery of agents that alter gap junction function exist: for instance, localized application of antisense or mimetic peptides that target Cx43 has been accomplished using pluronic gels and cell-specific nanogels[142,143]. Such strategies therefore have the potential to overcome the problems associated with off-target effects to make connexins potential candidates for further investigation in the treatment of joint disease.

1. Genetos, D. C., Kephart, C. J., Zhang, Y., Yellowley, C. E. & Donahue, H. J. Oscillating fluid flow activation of gap junction hemichannels induces ATP release from MLO-Y4 osteocytes. *J. Cell. Physiol.* **212**, 207–214 (2007).
2. Jiang, J. X. & Cherian, P. P. Hemichannels formed by connexin 43 play an important role in the release of prostaglandin E(2) by osteocytes in response to mechanical strain. *Cell Commun. Adhes.* **10**, 259–264 (2003).
3. Donahue, H. Gap junctions and biophysical regulation of bone cell differentiation. *Bone* **26**, 417–422 (2000).
4. Thi, M. M., Islam, S., Suadicani, S. O. & Spray, D. C. Connexin 43 and pannexin 1 channels in osteoblasts: who is the "hemichannel"? *J. Membr. Biol.* **245**, 401–409 (2012).
5. Willecke, K. *et al.* Structural and functional diversity of connexin genes in the mouse and human genome. *Biol. Chem.* **383**, 725–737 (2002).
6. Beyer, E. C., Paul, D. L. & Goodenough, D. A. Connexin 43: a protein from rat heart homologous to a gap junction protein from liver. *J. Cell Biol.* **105**, 2621–2629 (1987).
7. Kumar, N. M. & Gilula, N. B. Cloning and characterization of human and rat liver cDNAs coding for a gap junction protein. *J. Cell Biol.* **103**, 767–776 (1986).
8. Toyofuku, T. *et al.* Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes. *J. Biol. Chem.* **273**, 12725–12731 (1998).
9. Plotkin, L. I. & Bellido, T. Bisphosphonate-induced, hemichannel-mediated, anti-apoptosis through the Src/ERK pathway: a gap junction-independent action of connexin 43. *Cell Commun. Adhes.* **8**, 377–382 (2001).
10. Plotkin, L. I., Manolagas, S. C. & Bellido, T. Transduction of cell survival signals by connexin-43 hemichannels. *J. Biol. Chem.* **277**, 8648–8657 (2002).
11. Stains, J. P. & Civitelli, R. Connexins in the skeleton. *Semin. Cell Dev. Biol.* **50**, 31–39 (2016).
12. Loiselle, A. E., Jiang, J. X. & Donahue, H. J. Gap junction and hemichannel functions in osteocytes. *Bone* **54**, 205–212 (2013).
13. Kumar, N. M. & Gilula, N. B. The gap junction communication channel. *Cell* **84**, 381–388 (1996).
14. Martinez, A. D., Hayrapetyan, V., Moreno, A. P. & Beyer, E. C. Connexin 43 and connexin 45 form heteromeric gap junction channels in which individual components determine permeability and regulation. *Circ. Res.* **90**, 1100–1107 (2002).
15. Dallas, S. L., Prideaux, M. & Bonewald, L. F. The osteocyte: an endocrine cell and more. *Endocr. Rev.* **34**, 658–690 (2013).
16. Li, Z., Zhou, Z., Saunders, M. M. & Donahue, H. J. Modulation of connexin 43 alters expression of osteoblastic differentiation markers. *Am. J. Physiol. Cell Physiol.* **290**, C1248–C1255 (2006).
17. Grellier, M., Bareille, R., Bourget, C. & Amedee, J. Responsiveness of human bone marrow stromal cells to shear stress. *J. Tissue Eng. Regen Med.* **3**, 302–309 (2009).
18. Stains, J. P. & Civitelli, R. Gap junctions in skeletal development and function. *Biochim. Biophys. Acta* **1719**, 69–81 (2005).
19. Krüger, O. *et al.* Defective vascular development in connexin 45-deficient mice. *Development* **127**, 4179–4193 (2000).
20. Chaible, L. M., Sanches, D. S., Cogliati, B., Mennecier, G. & Dagli, M. L. Z. Delayed osteoblastic differentiation and bone development in Cx43 knockout mice. *Toxicol. Pathol.* **39**, 1046–1055 (2011).
21. Koval, M., Harley, J., Hick, E. & Steinberg, T. Connexin 46 is retained as monomers in a *trans*-Golgi compartment of osteoblastic cells. *J. Cell Biol.* **137**, 847–857 (1997).
22. Pacheco-Costa, R. *et al.* High bone mass in mice lacking Cx37 because of defective osteoclast differentiation. *J. Biol. Chem.* **289**, 8508–8520 (2014).
23. Paic, F. *et al.* Identification of differentially expressed genes between osteoblasts and osteocytes. *Bone* **45**, 682–692 (2009).
24. Reaume, A. G. *et al.* Cardiac malformation in neonatal mice lacking connexin 43. *Science* **267**, 1831–1834 (1995).
25. Lecanda, F. *et al.* Connexin 43 deficiency causes delayed ossification, craniofacial abnormalities, and osteoblast dysfunction. *J. Cell Biol.* **151**, 931–944 (2000).
26. Chung, D. J. *et al.* Low peak bone mass and attenuated anabolic response to parathyroid hormone in mice with an osteoblast-specific deletion of connexin 43. *J. Cell Sci.* **119**, 4187–4198 (2006).
27. Lima, F., Niger, C., Hebert, C. & Stains, J. P. Connexin 43 potentiates osteoblast responsiveness to fibroblast growth factor 2 via a protein kinase C-delta/Runx2-dependent mechanism. *Mol. Biol. Cell* **20**, 2697–2708 (2009).
28. Otto, F. *et al. Cbfa1*, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell* **89**, 765–771 (1997).
29. Buo, A. M., Tomlinson, R. E., Eidelman, E. R., Chason, M. & Stains, J. P. Connexin 43 and Runx2 interact to affect cortical bone geometry, skeletal development, and osteoblast and osteoclast function. *J. Bone Miner. Res.* **32**, 1727–1738 (2017).
30. Paznekas, W. A. *et al.* Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. *Am. J. Hum. Genet.* **72**, 408–418 (2003).
31. Paznekas, W. A. *et al. GJA1* mutations, variants, and connexin 43 dysfunction as it relates to the oculodentodigital dysplasia phenotype. *Hum. Mutat.* **30**, 724–733 (2009).
32. Laird, D. W. Syndromic and non-syndromic disease-linked Cx43 mutations. *FEBS Lett.* **588**, 1339–1348 (2014).
33. Chiba, H. *et al.* Relationship between the expression of the gap junction protein and osteoblast phenotype in a human osteoblastic cell line during cell proliferation. *Cell Struct. Funct.* **18**, 419–426 (1993).
34. Schiller, P. C., Roos, B. A. & Howard, G. A. Parathyroid hormone up-regulation of connexin 43 gene expression in osteoblasts depends on cell phenotype. *J. Bone Miner. Res.* **12**, 2005–2013 (1997).
35. Lecanda, F. *et al.* Gap junctional communication modulates gene expression in osteoblastic cells. *Mol. Biol. Cell* **9**, 2249–2258 (1998).
36. Watkins, M. *et al.* Osteoblast connexin 43 modulates skeletal architecture by regulating both arms of bone remodeling. *Mol. Biol. Cell* **22**, 1240–1251 (2011).
37. Bivi, N. *et al.* Cell autonomous requirement of connexin 43 for osteocyte survival: consequences for endocortical resorption and periosteal bone formation. *J. Bone Miner. Res.* **27**, 374–389 (2011).
38. Grimston, S. K., Watkins, M. P., Brodt, M. D., Silva, M. J. & Civitelli, R. Enhanced periosteal and endocortical responses to axial tibial compression loading in conditional connexin 43 deficient mice. *PLoS ONE* **7**, e44222 (2012).

39. Ilvesaro, J., Tavi, P. & Tuukkanen, J. Connexin-mimetic peptide Gap 27 decreases osteoclastic activity. *BMC Musculoskelet Disord.* **2**, 10 (2001).
40. Ilvesaro, J., Vaananen, K. & Tuukkanen, J. Bone-resorbing osteoclasts contain gap-junctional connexin-43. *J. Bone Miner. Res.* **15**, 919–926 (2000).
41. Ransjö, M., Sahli, J. & Lie, A. Expression of connexin 43 mRNA in microisolated murine osteoclasts and regulation of bone resorption *in vitro* by gap junction inhibitors. **303**, 1179–1185 (2003).
42. Jeansonne, B. G., Feagin, F. F., McMinn, R. W., Shoemaker, R. L. & Rehm, W. S. Cell-to-cell communication of osteoblasts. *J. Dent. Res.* **58**, 1415–1423 (1979).
43. Yellowley, C. E., Li, Z., Zhou, Z., Jacobs, C. R. & Donahue, H. J. Functional gap junctions between osteocytic and osteoblastic cells. *J. Bone Miner. Res.* **15**, 209–217 (2000).
44. Rawlinson, S., Pitsillides, A. & Lanyon, L. Involvement of different ion channels in osteoblasts' and osteocytes' early response to mechanical strain. *Bone* **19**, 609–614 (1996).
45. Duncan, R. L. & Turner, C. H. Mechanotransduction and the functional response of bone to mechanical strain. *Calcif. Tissue Int.* **57**, 344–358 (1995).
46. Ziambaras, K., Lecanda, F., Steinberg, T. H. & Civitelli, R. Cyclic stretch enhances gap junctional communication between osteoblastic cells. *J. Bone Miner. Res.* **13**, 218–228 (1998).
47. Alford, A. I., Jacobs, C. R. & Donahue, H. J. Oscillating fluid flow regulates gap junction communication in osteocytic MLO-Y4 cells by an ERK1/2 MAP kinase-dependent mechanism. *Bone* **33**, 64–70 (2003).
48. Cherian, P. P. *et al.* Effects of mechanical strain on the function of gap junctions in osteocytes are mediated through the prostaglandin EP2 receptor. *J. Biol. Chem.* **278**, 43146–43156 (2003).
49. Cheng, B. *et al.* Expression of functional gap junctions and regulation by fluid flow in osteocyte-like MLO-Y4 cells. *J. Bone Miner. Res.* **16**, 249–259 (2001).
50. Thi, M., Kojima, T., Cowin, S., Weinbaum, S. & Spray, D. Fluid shear stress remodels expression and function of junctional proteins in cultured bone cells. *Am. J. Physiol. Cell Physiol.* **284**, C389–C403 (2003).
51. Li, X. *et al.* Connexin 43 is a potential regulator in fluid shear stress-induced signal transduction in osteocytes. *J. Orthop. Res.* **31**, 1959–1965 (2013).
52. Saunders, M. M. *et al.* Gap junctions and fluid flow response in MC3T3-E1 cells. *Am. J. Physiol. Cell Physiol.* **281**, C1917–C1925 (2001).
53. Schirrmacher, K. & Bingmann, D. Effects of vitamin D3, 17β-estradiol, vasoactive intestinal peptide, and glutamate on electric coupling between rat osteoblast-like cells *in vitro*. *Bone* **23**, 521–526 (1998).
54. Taylor, A. F. *et al.* Mechanically stimulated osteocytes regulate osteoblastic activity via gap junctions. *Am. J. Physiol. Cell Physiol.* **292**, C545–C552 (2007).
55. Jørgensen, N. R. *et al.* Human osteoblastic cells propagate intercellular calcium signals by two different mechanisms. *J. Bone Miner. Res.* **15**, 1024–1032 (2000).
56. Bonewald, L. F. The amazing osteocyte. *J. Bone Miner. Res.* **26**, 229–238 (2011).
57. Batra, N. *et al.* Mechanical stress-activated integrin α5β1 induces opening of connexin 43 hemichannels. *Proc. Natl Acad. Sci. USA* **109**, 3359–3364 (2012).
58. Civitelli, R. Cell-cell communication in the osteoblast/osteocyte lineage. *Arch. Biochem. Biophys.* **473**, 188–192 (2008).
59. Grimston, S. K., Brodt, M. D., Silva, M. J. & Civitelli, R. Attenuated response to *in vivo* mechanical loading in mice with conditional osteoblast ablation of the connexin 43 gene (*Gja1*). *J. Bone Miner. Res.* **23**, 879–886 (2008).
60. Zhang, Y. *et al.* Enhanced osteoclastic resorption and responsiveness to mechanical load in gap junction deficient bone. *PLoS ONE* **6**, e23516 (2011).
61. Bivi, N. *et al.* Absence of Cx43 selectively from osteocytes enhances responsiveness to mechanical force in mice. *J. Orthop. Res.* **31**, 1075–1081 (2013).
62. Lloyd, S. A., Loiselle, A. E., Zhang, Y. & Donahue, H. J. Connexin 43 deficiency desensitizes bone to the effects of mechanical unloading through modulation of both arms of bone remodeling. *Bone* **57**, 76–83 (2013).
63. Grimston, S. K. *et al.* Connexin 43 deficiency reduces the sensitivity of cortical bone to the effects of muscle paralysis. *J. Bone Miner. Res.* **26**, 2151–2160 (2011).
64. Robling, A. G. *et al.* Mechanical stimulation of bone *in vivo* reduces osteocyte expression of Sost/sclerostin. *J. Biol. Chem.* **283**, 5866–5875 (2008).
65. Tu, X. *et al.* Sost downregulation and local Wnt signaling are required for the osteogenic response to mechanical loading. *Bone* **50**, 209–217 (2011).
66. Oyamada, M., Takebe, K. & Oyamada, Y. Regulation of connexin expression by transcription factors and epigenetic mechanisms. *Biochim. Biophys. Acta* **1828**, 118–133 (2013).
67. Robinson, J. A. *et al.* Wnt/β-catenin signaling is a normal physiological response to mechanical loading in bone. *J. Biol. Chem.* **281**, 31720–31728 (2006).
68. Xu, H. *et al.* Connexin 43 channels are essential for normal bone structure and osteocyte viability. *J. Bone Miner. Res.* **30**, 550–562 (2015).
69. Somoza, R. A., Welter, J. F., Correa, D. & Caplan, A. I. Chondrogenic differentiation of mesenchymal stem cells: challenges and unfulfilled expectations. *Tissue Eng. Part B Rev.* **20**, 596–608 (2014).
70. Eiberger, J. *et al.* Expression pattern and functional characterization of connexin 29 in transgenic mice. *Glia* **53**, 601–611 (2006).
71. Ralphs, J. R. *et al.* Regional differences in cell shape and gap junction expression in rat Achilles tendon: relation to fibrocartilage differentiation. *J. Anat.* **193**, 215–222 (1998).
72. Bruehlmann, S. B., Rattner, J. B., Matyas, J. R. & Duncan, N. A. Regional variations in the cellular matrix of the annulus fibrosus of the intervertebral disc. *J. Anat.* **201**, 159–171 (2002).
73. Hellio Le Graverand, M. P. *et al.* Formation and phenotype of cell clusters in osteoarthritic meniscus. *Arthritis Rheum.* **44**, 1808–1818 (2001).
74. Gruber, H. E., Ma, D., Hanley, E. N., Ingram, J. & Yamaguchi, D. T. Morphologic and molecular evidence for gap junctions and connexin 43 and 45 expression in annulus fibrosus cells from the human intervertebral disc. *J. Orthop. Res.* **19**, 985–989 (2001).
75. Knight, M. M., McGlashan, S. R., Garcia, M., Jensen, C. G. & Poole, C. A. Articular chondrocytes express connexin 43 hemichannels and P2 receptors - a putative mechanoreceptor complex involving the primary cilium? *J. Anat.* **214**, 275–283 (2009).
76. Mayan, M. D. *et al.* Human articular chondrocytes express multiple gap junction proteins: differential expression of connexins in normal and osteoarthritic cartilage. *Am. J. Pathol.* **182**, 1337–1346 (2013).
77. Schwab, W., Hofer, A. & Kasper, M. Immunohistochemical distribution of connexin 43 in the cartilage of rats and mice. *Histochem. J.* **30**, 413–419 (1998).
78. Donahue, H. J. *et al.* Chondrocytes isolated from mature articular cartilage retain the capacity to form functional gap junctions. *J. Bone Miner. Res.* **10**, 1359–1364 (1995).
79. Mayan, M. D. *et al.* Articular chondrocyte network mediated by gap junctions: role in metabolic cartilage homeostasis. *Ann. Rheum. Dis.* **74**, 275–284 (2015).
80. D'Andrea, P. & Vittur, F. Propagation of intercellular Ca^{2+} waves in mechanically stimulated articular chondrocytes. *FEBS Lett.* **400**, 58–64 (1997).
81. Schrobback, K., Klein, T. J. & Woodfield, T. B. F. The importance of connexin hemichannels during chondroprogenitor cell differentiation in hydrogel versus microtissue culture models. *Tissue Engineer. Part A* **21**, 1785–1794 (2015).
82. Loty, S., Foll, C., Forest, N. & Sautier, J. M. Association of enhanced expression of gap junctions with *in vitro* chondrogenic differentiation of rat nasal septal cartilage-released cells following their dedifferentiation and redifferentiation. *Arch. Oral Biol.* **45**, 843–856 (2000).
83. Willebrords, J., Maes, M., Crespo Yanguas, S. & Vinken, M. Inhibitors of connexin and pannexin channels as potential therapeutics. *Pharmacol. Ther.* **180**, 144–160 (2017).
84. Garcia, M. & Knight, M. M. Cyclic loading opens hemichannels to release ATP as part of a chondrocyte mechanotransduction pathway. *J. Orthop. Res.* **28**, 510–515 (2010).
85. Contreras, J. E. *et al.* Metabolic inhibition induces opening of unapposed connexin 43 gap junction hemichannels and reduces gap junctional communication in cortical astrocytes in culture. *Proc. Natl Acad. Sci. USA* **99**, 495–500 (2002).
86. Stout, C., Costantin, J., Naus, C. & Charles, A. Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels. *J. Biol. Chem.* **277**, 10482–10488 (2002).
87. Gomes, P., Srinivas, S. P., Van Driessche, W., Vereecke, J. & Himpens, B. ATP release through connexin hemichannels in corneal endothelial cells. *Invest. Ophthalmol. Vis. Sci.* **46**, 1208–1218 (2005).
88. Burnstock, G., Arnett, T. R. & Orriss, I. R. Purinergic signalling in the musculoskeletal system. *Purinerg. Signal* **9**, 541–572 (2013).
89. Graff, R. D., Lazarowski, E. R., Banes, A. J. & Lee, G. M. ATP release by mechanically loaded porcine chondrons in pellet culture. *Arthritis Rheum.* **43**, 1571–1579 (2000).
90. Zhang, J. *et al.* Connexin 43 hemichannels mediate small molecule exchange between chondrocytes and matrix in biomechanically-stimulated temporomandibular joint cartilage. *Osteoarthr. Cartilage* **22**, 822–830 (2014).
91. Groth, H. P. Cellular contacts in the synovial membrane of the cat and the rabbit: an ultrastructural study. *Cell Tissue Res.* **164**, 52541 (1975).
92. Dryll, A., Lansaman, J., Peltier, A. P. & Ryckewaert, A. Cellular junctions in normal and inflammatory human synovial-membrane revealed by tannic-acid and freeze-fracture. *Virchows Arch. A Pathol. Anat. Histol.* **386**, 293–302 (1980).
93. Kolomytkin, O. V. *et al.* Gap junctions in human synovial cells and tissue. *J. Cell. Physiol.* **184**, 110–117 (2000).
94. Capozzi, I., Tonon, R. & D'Andrea, P. Ca^{2+}-sensitive phosphoinositide hydrolysis is activated in synovial cells but not in articular chondrocytes. *Biochem. J.* **344**, 545–553 (1999).
95. D'Andrea, P., Calabrese, A. & Grandolfo, M. Intercellular calcium signalling between chondrocytes and synovial cells in co-culture. *Biochem. J.* **329**, 681–687 (1998).
96. Merrifield, P. A. & Laird, D. W. Connexins in skeletal muscle development and disease. *Semin. Cell Dev. Biol.* **50**, 67–73 (2016).
97. von Maltzahn, J., Euwens, C., Willecke, K. & Söhl, G. The novel mouse connexin 39 gene is expressed in developing striated muscle fibers. *J. Cell Sci.* **117**, 5381–5392 (2004).
98. Belluardo, N., Trovato-Salinaro, A., Mudò, G. & Condorelli, D. F. Expression of the rat connexin 39 (rCx39) gene in myoblasts and myotubes in developing and regenerating skeletal muscles: an *in situ* hybridization study. *Cell Tissue Res.* **320**, 299–310 (2005).
99. von Maltzahn, J., Wulf, V. & Willecke, K. Spatiotemporal expression of connexin 39 and 43 during myoblast differentiation in cultured cells and in the mouse embryo. *Cell Commun. Adhes.* **13**, 55–60 (2006).
100. von Maltzahn, J., Wulf, V., Matern, G. & Willecke, K. Connexin 39 deficient mice display accelerated myogenesis and regeneration of skeletal muscle. *Exp. Cell Res.* **317**, 1169–1178 (2011).
101. Dahl, E., Winterhager, E., Traub, O. & Willecke, K. Expression of gap junction genes, connexin 40 and connexin 43, during fetal mouse development. *Anat. Embryol.* **191**, 267–278 (1995).
102. Balogh, S., Naus, C. C. & Merrifield, P. A. Expression of gap junctions in cultured rat L6 cells during myogenesis. *Dev. Biol.* **155**, 351–360 (1993).
103. Proulx, A., Merrifield, P. A. & Naus, C. Blocking gap junctional intercellular communication in myoblasts inhibits myogenin and MRF4 expression. *Dev. Genet.* **20**, 133–144 (1997).
104. Yamanouchi, K., Yada, E., Ishiguro, N. & Nishihara, M. 18α-glycyrrhetinic acid induces phenotypic changes of skeletal muscle cells to enter adipogenesis. *Cell. Physiol. Biochem.* **20**, 781–790 (2007).
105. Plotkin, L. I., Davis, H. M., Cisterna, B. A. & Sáez, J. C. Connexins and pannexins in bone and skeletal muscle. *Curr. Osteoporosis Rep.* **15**, 326–334 (2017).
106. Araya, R. *et al.* Expression of connexins during differentiation and regeneration of skeletal muscle: functional relevance of connexin 43. *J. Cell Sci.* **118**, 27–37 (2005).
107. Cea, L. A. *et al.* De novo expression of connexin hemichannels in denervated fast skeletal muscles leads to atrophy. *Proc. Natl Acad. Sci. USA* **110**, 16229–16234 (2013).
108. Cea, L. A. *et al.* Fast skeletal myofibers of mdx mouse, model of Duchenne muscular dystrophy, express connexin hemichannels that lead to apoptosis. *Cell. Mol. Life Sci.* **73**, 2583–2599 (2016).
109. Muramatsu, T. *et al.* Reduction of connexin 43 expression in aged human dental pulp. *Int. Endod J.* **37**, 814–818 (2004).

110. Yeh, H. I. *et al.* Age-related alteration of gap junction distribution and connexin expression in rat aortic endothelium. *J. Histochem. Cytochem.* **48**, 1377–1389 (2000).
111. Xie, H. Q. & Hu, V. W. Modulation of gap junctions in senescent endothelial cells. *Exp. Cell Res.* **214**, 172–176 (1994).
112. Boengler, K., Heusch, G. & Schulz, R. Connexin 43 and ischemic preconditioning: effects of age and disease. *Exp. Gerontol.* **41**, 485–488 (2006).
113. Genetos, D. C., Zhou, Z., Li, Z. & Donahue, H. J. Age-related changes in gap junctional intercellular communication in osteoblastic cells. *J. Orthop. Res.* **30**, 1979–1984 (2012).
114. Kastner, M. *et al.* Complex interventions can increase osteoporosis investigations and treatment: a systematic review and meta-analysis. *Osteoporos Int.* **7**, 407 (2017).
115. Schiller, P. C., D'Ippolito, G., Balkan, W., Roos, B. A. & Howard, G. A. Gap-junctional communication mediates parathyroid hormone stimulation of mineralization in osteoblastic cultures. *Bone* **28**, 38–44 (2001).
116. Plotkin, L. I. *et al.* Connexin 43 is required for the anti-apoptotic effect of bisphosphonates on osteocytes and osteoblasts *in vivo*. *J. Bone Miner. Res.* **23**, 1712–1721 (2008).
117. Loiselle, A. E. *et al.* Specific biomimetic hydroxyapatite nanotopographies enhance osteoblastic differentiation and bone graft osteointegration. *Tissue Eng. Part A* **19**, 1704–1712 (2013).
118. Lloyd, S. A., Loiselle, A. E., Zhang, Y. & Donahue, H. J. Shifting paradigms on the role of connexin 43 in the skeletal response to mechanical load. *J. Bone Miner. Res.* **29**, 275–286 (2014).
119. Malemud, C. J. Biologic basis of osteoarthritis: state of the evidence. *Curr. Opin. Rheumatol* **27**, 289–294 (2015).
120. Goldring, M. B. & Goldring, S. R. Osteoarthritis. *J. Cell. Physiol.* **213**, 626–634 (2007).
121. arcOGEN Consortium *et al.* Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. *Lancet* **380**, 815–823 (2012).
122. Hellio Le Graverand, M. P. *et al.* The cells of the rabbit meniscus: their arrangement, interrelationship, morphological variations and cytoarchitecture. *J. Anat.* **198**, 525–535 (2001).
123. Miyamoto, Y. *et al.* A functional polymorphism in the 5′ UTR of GDF5 is associated with susceptibility to osteoarthritis. *Nat. Genet.* **39**, 529–533 (2007).
124. Chapman, K. *et al.* A meta-analysis of European and Asian cohorts reveals a global role of a functional SNP in the 5′ UTR of GDF5 with osteoarthritis susceptibility. *Hum. Mol. Genet.* **17**, 1497–1504 (2008).
125. Chatterjee, B. *et al.* BMP regulation of the mouse connexin 43 promoter in osteoblastic cells and embryos. *Cell Commun. Adhes.* **10**, 37–50 (2003).
126. Coleman, C. M., Loredo, G. A., Lo, C. W. & Tuan, R. S. Correlation of GDF5 and connexin 43 mRNA expression during embryonic development. *Anat. Rec. A Discov. Mol. Cell Evol. Biol.* **275**, 1117–1121 (2003).
127. Marino, A. A. *et al.* Increased intercellular communication through gap junctions may contribute to progression of osteoarthritis. *Clin. Orthop. Relat. Res.* **422**, 224–232 (2004).
128. Giepmans, B. N. G. Role of connexin 43-interacting proteins at gap junctions. *Adv. Cardiol.* **42**, 41–56 (2006).
129. Giepmans, B. N. *et al.* Gap junction protein connexin-43 interacts directly with microtubules. *Curr. Biol.* **11**, 1364–1368 (2001).
130. Moorby, C. & Patel, M. Dual functions for connexins: Cx43 regulates growth independently of gap junction formation. *Exp. Cell Res.* **271**, 238–248 (2001).
131. Gago-Fuentes, R. *et al.* The C-terminal domain of connexin 43 modulates cartilage structure via chondrocyte phenotypic changes. *Oncotarget* **7**, 73055–73067 (2016).
132. Gago-Fuentes, R. *et al.* Proteomic analysis of connexin 43 reveals novel interactors related to osteoarthritis. *Mol. Cell. Proteom.* **14**, 1831–1845 (2015).
133. Homandberg, G. A., Meyers, R. & Williams, J. M. Intraarticular injection of fibronectin fragments causes severe depletion of cartilage proteoglycans *in vivo*. *J. Rheumatol.* **20**, 1378–1382 (1993).
134. Homandberg, G. A. & Hui, F. Association of proteoglycan degradation with catabolic cytokine and stromelysin release from cartilage cultured with fibronectin fragments. *Arch. Biochem. Biophys.* **334**, 325–331 (1996).
135. Benito, M. J., Veale, D. J., FitzGerald, O., van den Berg, W. B. & Bresnihan, B. Synovial tissue inflammation in early and late osteoarthritis. *Ann. Rheum. Dis.* **64**, 1263–1267 (2005).
136. Bondeson, J. Are we moving in the right direction with osteoarthritis drug discovery? *Expert Opin. Ther. Targets* **15**, 1355–1368 (2011).
137. Kolomytkin, O. V. *et al.* IL-1β-induced production of metalloproteinases by synovial cells depends on gap junction conductance. *Am. J. Physiol. Cell Physiol.* **282**, C1254–C1260 (2002).
138. Tonon, R. & D'Andrea, P. Interleukin-1β increases the functional expression of connexin 43 in articular chondrocytes: evidence for a Ca^{2+}-dependent mechanism. *J. Bone Miner. Res.* **15**, 1669–1677 (2000).
139. Tonon, R. & D'Andrea, P. The functional expression of connexin 43 in articular chondrocytes is increased by interleukin 1β: evidence for a Ca^{2+}-dependent mechanism. *Biorheology* **39**, 153–160 (2002).
140. Gupta, A. *et al.* Connexin 43 enhances the expression of osteoarthritis-associated genes in synovial fibroblasts in culture. *BMC Musculoskelet Disord.* **15**, 425 (2014).
141. Tsuchida, S. *et al.* Silencing the expression of connexin 43 decreases inflammation and joint destruction in experimental arthritis. *J. Orthop. Res.* **31**, 525–530 (2013).
142. Grek, C. L., Rhett, J. M. & Ghatnekar, G. S. Cardiac to cancer: Connecting connexins to clinical opportunity. *FEBS Lett.* **588**, 1349–1364 (2014).
143. Baklaushev, V. P. *et al.* Treatment of glioma by cisplatin-loaded nanogels conjugated with monoclonal antibodies against Cx43 and BSAT1. *Drug Delivery* **22**, 276–285 (2014).

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SCIENCE AND SOCIETY

The RA-MAP Consortium: a working model for academia–industry collaboration

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Abstract | Collaboration can be challenging; nevertheless, the emerging successes of large, multi-partner, multi-national cooperatives and research networks in the biomedical sector have sustained the appetite of academics and industry partners for developing and fostering new research consortia. This model has percolated down to national funding agencies across the globe, leading to funding for projects that aim to realise the true potential of genomic medicine in the 21st century and to reap the rewards of 'big data'. In this Perspectives article, the experiences of the RA-MAP consortium, a group of more than 140 individuals affiliated with 21 academic and industry organizations that are focused on making genomic medicine in rheumatoid arthritis a reality are described. The challenges of multi-partner collaboration in the UK are highlighted and wide-ranging solutions are offered that might benefit large research consortia around the world.

Over the past few years, the relative failure by scientists to reap the benefits of the genomics revolution, along with the pressing challenges and perceived opportunities that accompany the analysis of 'big data', have led to a concerted drive towards the development of cooperative academia–industry initiatives across a range of diseases[1,2]. This move towards consortia acknowledges the need to advance health care initiatives in a systematic way and places emphasis on the collective harnessing of knowledge, resources and expertise in ways that are both complementary and mutually beneficial to all parties[3–6]. Central to these initiatives has been the creation of nonexclusive consortia in pre-competitive areas of research (research aimed at the generation of new knowledge) that capitalize on expertise from multiple sources and reward all partners for their contributions[7,8]. In this Perspectives article, we describe the experience of setting up the RA-MAP consortium, a multi-partner academia–industry partnership, and highlight some of the challenges we faced and solutions we adopted to successfully direct a collaborative consortium focused on rheumatoid arthritis (RA).

Stratified medicine

Stratified medicine has been defined in a wide variety of ways[9]: the Association of the British Pharmaceutical Industry (ABPI) defines it as "the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a particular treatment"[10]. The term has also been used interchangeably with precision, personalised or P4 medicine[9,11]. In line with these definitions, and in an effort to realise the full potential of stratified medicine[12], funding bodies have sought to support research that provides new insights into disease mechanisms, enabling the tailoring of existing treatments to individuals and paving the way for the development of new treatments, diagnostic methods and care pathways[13,14].

Arguably, physicians have been practising precision medicine for centuries, individualizing therapy on the basis of personalized clinical assessment in combination with rudimentary investigations such as haematological and biochemical profiles, as well as radiographic imaging and histopathological investigations. Contemporary concepts of tailoring therapy to specific patient subgroups have been driven by a growing appreciation of pathway biology, in which common clinical syndromes are underpinned by aberrations in specific molecular and cellular processes, and the development of sophisticated laboratory tools to define these distinct pathways[15,16]. Sequencing and annotation of the human genome, coupled with advances in next generation sequencing technology, have been at the forefront of stratified medicine, enabling researchers to uncover molecular associations with specific disease phenotypes[17,18], drug responses and drug toxicities[19], as well as to define novel pathogenic molecular pathways that underpin disease risk[20]. Genomic fingerprinting, along with transcriptomics, epigenomics, proteomics and metabolomics, are just a few of the 'omics' technologies that enable a truly systematic and unbiased approach to understanding the molecular basis of disease. The omics revolution is generating data on an unprecedented

Box 1 | **Establishing a successful stratified medicine consortium**

Several key elements are required when setting up an academia–industry partnership.

- A consensus on the importance of identifying common disease pathways.
- Engaged industrial partners with emerging drug pipelines.
- Existing efficacious therapies that might be suitable for repurposing.
- An urgent need for disease phenotyping and biomarker-based patient stratification.
- The need for a better understanding of the relationship between clinical and pathological phenotypes.
- The availability of emerging technologies to redefine disease subtypes at a molecular and cellular level.
- Regional or national colocalisation of partners.
- A rich patient bioresource.
- Access to clinical research infrastructures, for example the National Health Service and National Institute for Health Research in the UK.
- Enthusiastic support from patient groups.

scale[21], leading to the need for major advances in informatics, data integration, data science and methods for analysing big data, a set of disciplines that are often captured under the umbrella term of 'systems biology and bioinformatics'[22]. The overriding goal of stratified medicine is early, precise diagnosis of disease and early therapeutic intervention, applying 'the five rights' of medication use (a concept adapted from standards for safe medication practices): the right patient, the right drug, the right time, the right dose and the right route of administration[23]. A future goal of stratified medicine would be to use these data to define the preclinical disease state with a view to personalized preventive medicine. Such big data approaches are underpinned by the belief that the classical clinical phenotype of a disease such as RA is actually composed of a variety of distinct molecular endotypes[24], each one predicated on inherited, environmental and stochastic differences between patients.

Nowhere has stratified medicine had a greater effect to date than in cancer; genotyping patients for *BRCA* mutations[25], screening patients for gene translocations[26,27] and analysis of expression of *ERBB2* combined with *in situ* tissue typing in patients with breast cancer[28,29], for example, have transformed therapy through a deeper understanding of oncogenesis at the molecular level. This deeper knowledge of oncogenesis has led to cancer prevention and to the rational design of small molecule tyrosine kinase inhibitors and monoclonal antibodies, with proof-of-concept being established during clinical trials[30,31]. The stratification of patients according to their immune phenotype is also progressing rapidly in the field of checkpoint inhibitor therapy[32–34]. On the basis of these advances, there has been considerable interest in the past few years in applying these principles to other diseases that might benefit from a similar experimental approach. An academia–industry collaboration designed along the lines of the contemporary concepts outlined above would provide a strong platform from which to deliver such an ambitious programme of work.

MRC–ABPI-funded programmes

In 2008, the UK Medical Research Council (MRC) published a strategic review of human immunology, which provided a roadmap for building capacity, for the creation of an interdisciplinary environment and for an increase in connectivity between institutions and sectors[35]. In 2009, in response to the last of these points, the MRC Human Immunology and Inflammation Initiative identified obstacles to closer academia–industry interaction, solutions to which included improved networking, improved access to human tissue samples and improved support for clinical researchers. Two disease-focused workshops, covering RA and chronic obstructive pulmonary disease, were held in 2010 to begin to address these important issues. The rationale for selecting RA as a model disease for this approach was driven by a combination of UK expertise in the field and specific unmet clinical needs and knowledge gaps for the disease. These unmet needs included robust strategies for the stratification of patients and suitable biomarkers to inform such stratification, technology to predict responses to specific therapies and molecular and cellular signatures to identify a state of true biological remission. At these workshops, the discussions focused on approaches to stratified medicine and placed particular emphasis on prioritising research into disease pathways and on how an ambitious and incisive programme of research might best be delivered. Key requirements for establishing a successful consortium were highlighted during these discussions and are summarised in BOX 1. In 2011, the MRC–ABPI Inflammation and Immunity Initiative was formally launched in an attempt to address some of the specific unmet needs of patients with RA.

The immunological concept. After considering the requirements listed in BOX 1, the RA-focused working group concluded that the missing element was a full understanding of the immune dysregulation that underpins RA. If the immunology of the disease could be better characterized, it followed that biomarkers could then be developed to stratify patients with the disease and to inform therapy choices. Theoretically, these cellular and molecular tools could be integrated into an immunological toolkit that would consist of a combination of clinical and laboratory parameters measured in patients with early RA that could be used to predict clinical responses to DMARDs, to monitor biological responses to therapy and to define a true state of biological remission. This proposal was predicated on the following principles: the healthy immune system is associated with an immunological fingerprint that can be defined by serum, cellular and/or molecular signatures in peripheral blood; RA is associated with detectable perturbations of the immune system at very early stages of disease[36] that can be used to distinguish subsets of patients; restoration of immune health in patients with RA might be inducible by therapies that target these perturbations; and clinical remission is associated with a biological state that might have similarities to a healthy immune system. It was thought that, if successful, such an approach could have an immediate effect on our understanding of a broad range of immune-mediated inflammatory diseases.

The RA-MAP Consortium. In 2012, following a successful funding application focused on the principles described above, the Rheumatoid Arthritis MRC–ABPI (RA-MAP) Consortium was conceived. The consortium has since expanded to include 11 industry partners and 10 UK academic partners who share a deep-rooted enthusiasm for translational

science in the field of immunology and inflammation in the pre-competitive space (Supplementary information S1 (figure)). Membership of the consortium reflects contributions and commitments by various partners to genomic medicine, genetics and immunology and inflammation biology; expertise in immune phenotyping, metabolomics and proteomics; clinical expertise in assembling and curating patient cohorts and deep clinical phenotyping; and centres of excellence in experimental medicine with a focus on early inflammatory arthritis. Unusually, the consortium was established with a close relationship between the funding body and the researchers, which created a new paradigm for collaborative working.

The RA-MAP Consortium has similarities to other research networks that focus on research into rheumatic diseases (TABLE 1), including the Accelerating Medicines Partnership (AMP) RA and systemic lupus erythematosus network, a partnership that was launched in 2014. This US network seeks to define new therapies and diagnostic technologies for rheumatic autoimmune diseases by utilizing a systems-level understanding of transcriptomic signatures derived from synovial, kidney and skin tissues. Along similar lines, the European Union (EU)-funded PRECISESADS consortium focuses on redefining autoimmune diseases at a molecular level (TABLE 1). In operational terms, EU consortia have benefited considerably from the experiences of previous academia–industry partnerships, such as AutoCure, MASTERSWITCH and Be the Cure (BTCure) (TABLE 1). The longevity of these programmes has served to fuel the productivity of research and to facilitate collaborations between public sector and private sector organizations. Since its inception, the MRC Stratified Medicine strategic initiative has also supported several other consortia that focus on immune-mediated inflammatory diseases (TABLE 1).

A key challenge for the RA-MAP Consortium was to harness the synergistic skill sets of pharmaceutical companies, biotechnology companies and academic partners to develop a programme of activities that would address each specific scientific goal. To do so required the establishment of a sizeable new inception cohort of treatment-naive patients with RA who had a relatively short duration of symptoms and were willing to provide biological samples. This cohort of patients was called Towards a Cure for Early RA (TACERA), and the samples from these patients provided the substrate for cutting-edge analytical techniques. The next step was to apply innovative systems approaches to analyse and assemble the data from multiple omics platforms into predictive algorithms, with the ultimate aim being the development of a set of informative assays that would provide a toolkit to facilitate patient stratification in a clinical setting (FIG. 1). A cohort of healthy individuals who were followed longitudinally following vaccination with a neoantigen was enrolled to provide a suitable control population with which to compare the signatures of immune dysregulation identified in patients with RA.

Although each industry partner had their own strategic reasons for joining the consortium, the overriding motivation of these companies to partner with academia was the shared recognition that this study would generate data in a real-world population of patients with RA that could improve our understanding of the subsets of disease and associated immunological phenotypes that characterize the early phase of RA. Working collaboratively with companies and various academic centres was thought to increase the chances of producing clinically relevant knowledge about opportunities for intervention and indicators of response in these patients. To achieve these goals, the RA-MAP Consortium divided its tasks into various research work packages (see Supplementary information S2 (figure)).

For the remainder of this Perspectives article we aim to describe some of the operational and scientific challenges that are faced by large research consortia and to highlight solutions that can be adopted to overcome such challenges with reference to specific examples from our experience with the RA–MAP Consortium.

Table 1 | **Academia–industry consortia in immune-mediated inflammatory diseases**

Consortium	Contributors	Website
International consortia		
AMP RA and SLE network	• NIH • FDA • Ten industry partners • Multiple academic research units	https://amp-ralupus.stanford.edu/
PRECISESADS consortium	• Five EFPIA partners • Two SMEs • 21 academic partners	http://www.precisesads.eu/
AutoCure	• Six EFPIA partners • 20 academic partners	http://www.crb.uu.se/research/projects/autocure/
MASTERSWITCH	• Four SMEs • 15 academic partners	http://cordis.europa.eu/result/rcn/147588_en.html
Be the Cure	• Nine EFPIA partners • Six SMEs • 24 academic partners	http://btcure.eu
Rheuma Tolerance for Cure	• Six EFPIA partners • Two SMEs • 12 academic partners	http://cordis.europa.eu/project/rcn/211964_en.html
MRC Stratified Medicine consortia		
MATURA	• Ten industry partners • 12 academic partners • Jointly funded by ARUK	http://www.matura.whri.qmul.ac.uk
PSORT	• Seven industry partners • 12 academic and NHS partners	http://www.psort.org.uk
MASTERPLANS	• Four industry partners • Eight academic and NHS partners	http://www.lupusmasterplans.org/home.html

AMP, Accelerating Medicines Partnership; ARUK, Arthritis Research UK; EFPIA, European Federation of Pharmaceutical Industries and Associations; FDA, US Food and Drug Administration; NIH, National Institutes of Health; NHS, National Health Service; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SME, small or medium sized enterprise.

Challenges and solutions

Some of the key challenges that are faced by academia–industry consortia are summarised in BOX 2. Further insights and suggested solutions derived from the experience of the RA-MAP Consortium are described in detail below.

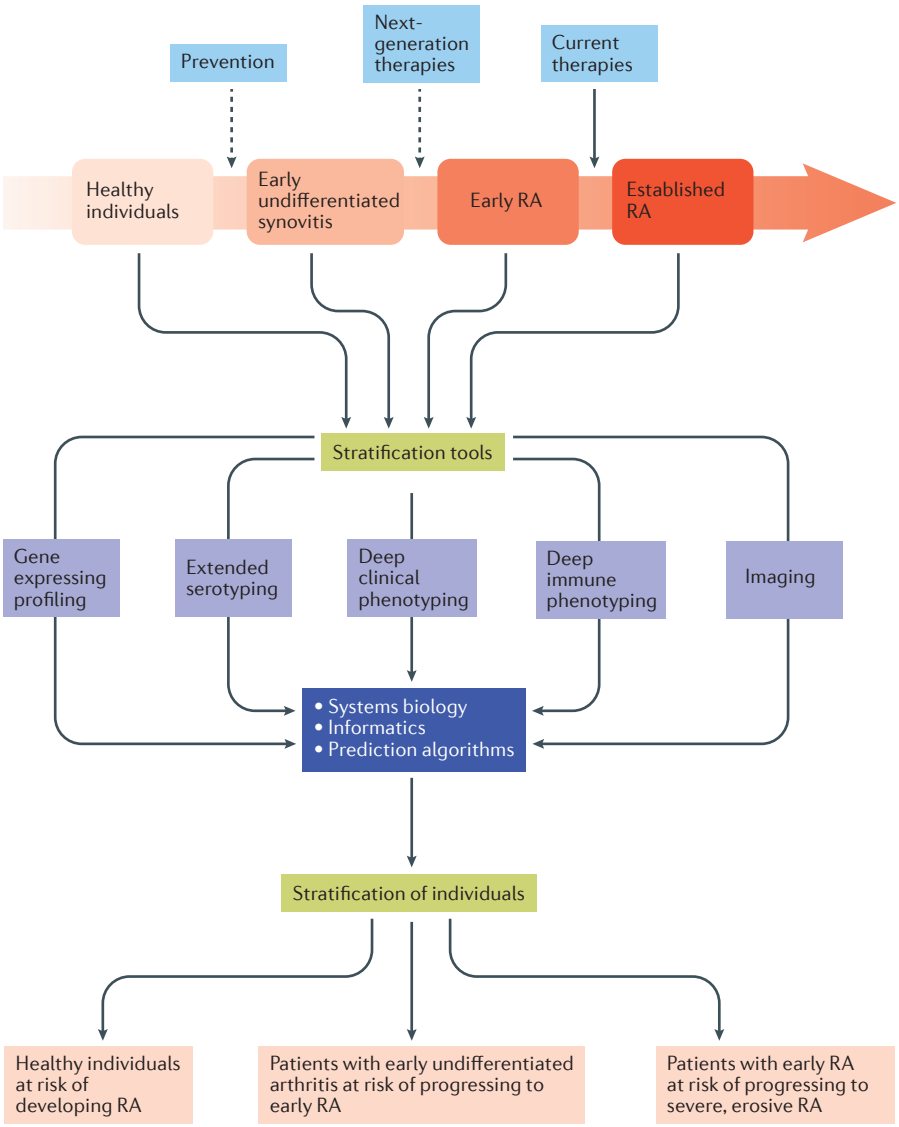

Figure 1 | **Stratification of patients with rheumatoid arthritis.** Stratification of patients with rheumatoid arthritis (RA) can occur at several points during the natural history of the disease. Stratification describes a process of characterising subgroups of patients according to distinct clinical, cellular and molecular features (or endotypes) using any combination of parameters. Multiple platforms can be adopted to stratify patients throughout the disease course, including serotyping, clinical and immunological phenotyping, genotyping and imaging.

The contract. A major challenge for any consortium is one of scale. In any group of academic and industry partners who each have distinct agendas, experiences and governance structures, individual partners will have different expectations. This discrepancy requires sympathetic management so that the ambitions of all parties can be met. Agreement of the scientific goals of the consortium provides a common purpose, for which each partner can identify their potential contributions and resource provision. Tangible benefits for industry partners are central to success and to the sustainable engagement of such partners; each company will value research 'currency' in a different way, but good examples might include access to deeply phenotyped cohorts of patients, access to downstream data and sharing of samples among partners. Interactions between and operations involving multiple institutions require a set of clear ground rules that go beyond a 'terms of reference' template. One possible solution is the consortium agreement, which provides an operating framework that emphasizes the obligations and responsibilities of leadership and membership and contains guidelines about the transfer and use of materials, liabilities and indemnity of each party, details of project management and data management practices including data protection and, importantly, publicity, publication and intellectual property rights. In essence, the agreement needs to be simple, pragmatic and a point of reference for the lifetime of the consortium and beyond.

Who owns the data? Reaching agreement over data protection and ownership can be a major challenge for research consortia because priorities and expectations can vary between the private and public sectors, notwithstanding the nuances that research in the pre-competitive space can offer. Nonetheless, this is an area in which the experience of industry can add value to a consortium, by helping to define relevant background to the project, supporting registration and protection of intellectual property rights arising from the data, filing and prosecuting patent applications or assisting in actions relating to infringement of intellectual property rights. In return, academic partners might agree to grant worldwide non-exclusive licenses to any industry partner to use the results of experiments and intellectual property for commercial purposes, taking into account the relative contribution made to the consortium by that industry partner. Members of the RA-MAP Consortium learned that much time can be saved, and barriers promptly overcome, by facilitating frequent, robustly managed communication between the intellectual property and technology transfer offices of each partner from the very outset.

How can industry partners contribute? Resource frameworks differ greatly depending on the scale and context of the research programme and the funding agency involved. For example, industry partners might be required to pledge specific levels of support, such as in-kind contributions, contributions of skilled personnel, funding for specific research projects or provision of access to technology platforms. Such has been the approach of the EU Framework 7 and Horizon 2020 programmes with respect to matched contributions from European Federation of Pharmaceutical Industries and Associations (EFPIA) partners[37]. Commitment to provide matched-funding from the outset has obvious advantages but, although these ground rules might not apply to all consortia, there are other

imaginative ways that industry partners can support the research agenda. The RA-MAP Consortium has benefited greatly from the patient-level data, advice on the setup of and study operations for the TACERA study, omics platforms, advice on the management of informatics and bioinformatics and statistical analysis that were provided by industry partners.

Consortium operations. Concepts of project management differ widely across sectors, yet robust management can determine the success or failure of a project. So, what are the options? Experience suggests that oversight of multi-partner projects can be greatly facilitated by a small executive Consortium Management Board that is co-chaired by industry and academia principal investigators. This board might take responsibility for coordinating activities and for reporting progress to the funder. A larger Project Steering Group, comprising representatives of all consortium partners, can operate as the decision-making body, using a legally binding consortium agreement as its terms of reference. Investment in full-time project managers with experience in both academia and industry can reap dividends. As the 'operators of operations', project managers are essential for organizing meetings and maintaining a sharp focus on project timelines, deliverables and milestones, as well as for the robust management of high-risk work packages, and are increasingly appreciated as vital assets in the academic setting. Infusing a project with a momentum that will last for its lifetime can be critical to success — an exemplar operating structure is illustrated in Supplementary information S3 (figure).

Box 2 | **Challenges faced by research consortia and possible solutions**

Agreement as to the terms of reference and ground rules for consortium operations
Generate a contract or consortium agreement with input from the contract and legal teams of all partners from the outset.

Data ownership
In any pre-competitive project, data can be shared and intellectual property arrangements can be addressed directly in the consortium agreement.

Industry contributions
Contributions from industry partners should be agreed from the start of the project. Examples of contributions should be provided that cover the areas of specific interest or expertise of each partner.

Project management
Management structures are essential and part of 'normal business' for industry partners. Capitalize on private sector expertise to establish lean, functional committees with clear terms of reference. Invest in a project manager, ideally with both academic and industry experience.

Managing staff turnover
Anticipate and redistribute resources to support the training of incoming technical and research staff; close liaison with industry partners to identify new colleagues with relevant skills and experience is essential.

Building a strong collaborative ethos
Identify areas of expertise and establish working groups made up of individuals from across all sectors who share common goals and who will commit to regular teleconference meetings.

Recruiting, site approval and set-up
Engage contract research organizations to support activities such as coordinating the acquisition of documentation for timely site-specific regulatory approval.

Quality control
Quality control applies as much to study protocols and standard operating procedures as it does to sample acquisition, processing and storage and to data analysis; procurement should be robust and outward-looking if the necessary expertise does not exist within the consortium.

Data analysis
Invest in state-of-the-art data warehouse capabilities and facilitate access by all parties. Define research priorities and construct a mutually agreed data analysis plan. Frequent opportunities for all partners to discuss results are essential to maintain momentum.

Publication
Agree to a publication policy and plan that provides shared authorship, where appropriate, and recognizes the contributions of the extended network of investigators.

Scientific review of milestones
Project reviews should be agreed with the funding organization, as appropriate, but should be regular, robust and led by an independent expert advisory committee and chair.

Coordinating biological sampling at multiple sites. Traditionally, the acquisition of an extended portfolio of samples, including intensive sampling over short periods of time, has been the remit of small, single-centre experimental medicine studies. Accredited centres specializing in phase I clinical trials and contract research organizations have streamlined this process over several decades, facilitated by the proximity of patients to the lab, short times from venesection to processing of samples and tried and tested standard operating procedures (SOPs) for processing, storing and analysing fresh samples. Large, multi-centre studies present a challenge in this regard, necessitating sizeable efforts to harmonize the acquisition, processing and storage of prospectively acquired biological samples, and compromises in terms of sample range and assay complexity. Sampling is often limited in such multi-centre studies to the monitoring of drug safety using local accredited clinical laboratories.

To address the challenge of collecting samples from multiple sites, the RA-MAP Consortium established a hub-and-spoke network of seven academic laboratory hubs across England and Scotland that serve 28 patient-recruiting centres. This approach enabled the transportation of study samples from any patient-recruiting site to a lab within 4 hours of venesection. The requirements for sample transport, and for subsequent processing and storage, were clearly documented in study SOPs and protocols, with each step of the sample transport process carefully logged by study staff. Specifically designed sample tracking and logging software was placed in each of the hub laboratories along with the necessary hardware, including barcode scanners. SOPs for complex sample processing were developed by the relevant partners, scrutinized by industry partners, and refined before participant recruitment. This approach enabled high quality, barcoded aliquots of serum, peripheral blood cells, whole-blood RNA, RNA from lymphocyte and monocyte subsets purified in each laboratory, genomic DNA and urine to be processed and stored (Supplementary information S4 (figure)).

Combined input from academic and industry partners can ensure that sampling protocols are optimized to support immunophenotyping, as well as metabolomic, proteomic and transcriptomic analyses. In addition, sample procurement of this magnitude requires sample storage that facilitates long-term access to samples by the wider research community. Well-funded national repositories are ideally suited to provide this platform; in the UK, the UK Biobank provides such a resource.

Quality control. By centralizing sample analysis, single-centre studies can ensure the consistency and quality of sample processing and analysis of fresh material. However, when a broad portfolio of analytical platforms, analysis and expertise is required, there are several pragmatic approaches that can be adopted. Analysing all samples at a single sitting has obvious advantages, especially for transcriptomics, proteomics and metabolomics; when performing such assays at scale (for example, RNA extraction and microarray analysis), outsourcing can prove to be both cost effective and scientifically justifiable. A particular challenge for multi-centre studies is flow cytometric analysis, because cell staining protocols vary widely and hardware and machine settings can dramatically alter immune phenotypes, not to mention the varying expression profiles generated by different antibodies and fluorophores. To address this challenge, aliquots of cryopreserved peripheral blood cells can be distributed to designated laboratories that have expertise in the deep phenotyping of a single leukocyte subset. Flow cytometer configurations can be harmonized and batches of fluorescence-conjugated monoclonal antibodies can be purchased in bulk and distributed to each centre to minimize experimental variability across sites and between assays. In cases when samples are evaluated by flow cytometry at multiple time-points, additional measures can be adopted to minimize batch effects (for example, by applying corrections using standard tools such as COMBAT[38]).

Curating the data. Data are one of the defining metrics for determining the success of a consortium. Study participant data is often derived from multiple sources, especially when combining clinical, laboratory, imaging and omics datasets. As an example, the RA-MAP Consortium oversaw the recruitment of an inception cohort of patients with RA (participants in the TACERA study), who were followed from first presentation for up to 18 months, accumulating >1,280 baseline and follow-up visits from 275 study participants. The scale of the programme and the breadth and depth of data acquired necessitated investment in data cleaning, curating and storage, in accordance with data protection guidelines and sharing and communication policies, which needed to comply with requirements for patient confidentiality on the one hand while facilitating data analysis on the other. For the TACERA study, data were securely transferred and pseudo-anonymised using the OpenPseudonymiser package before undergoing a curation process, which included data integrity checks and semantic normalization. The curated and reformatted data were uploaded to TranSMART, a data warehouse that enables data access, visualisation, exploration and download to all members of the consortium (Supplementary information S5 (figure)). The local platform of TranSMART belonging to the RA-MAP Consortium has provided service to 82 users from multiple organizations and stores 37GB of data on the MRC eMedLab cloud computing facility, offering high performance computing capacity, a solution for long-term data sustainability and an appropriate environment for future meta-analyses by the rheumatology and immune-mediated inflammatory disease research communities.

Analysis of multi-omic data. When dealing with large volumes of data, challenges arise beyond storage. The RA-MAP Consortium's portfolio of studies generated approximately 40 million analysis-ready data points from approximately 1 billion raw data points derived from more than 5,721 patient samples. The results of each omics platform investigation were stored in the TranSMART data warehouse, which provided an integrated view of omics platforms and linked clinical phenotypes, alongside a highly curated selection of pre-existing public data. TranSMART was chosen as it provided the RA-MAP Consortium and their partners with a unified, secure and, critically, sustainable research environment that offered on-board analytical capacity (including additional plugins such as SmartR[39]), data export and an R application programming interface, which enabled the use of a broad range of systems biology and machine learning methods for biomarker discovery.

Encouraging a sense of ownership of the data among all members of a consortium and overseeing the analysis by multiple parties require robust management. Agreement between partners and a clear alignment of goals between clinicians and the analytical teams, which might comprise biostatisticians, bioinformaticians and systems biologists from multiple partners, are essential for sustaining research momentum, maximizing output and for maintaining focus on pre-defined clinical questions. The RA-MAP Consortium found the adoption of a series of 'lab meeting'-style teleconferences to be particularly productive. During these meetings, bioinformaticians could discuss the analysis of data on individual platforms and systems biologists could direct overall data integration while at the same time retaining a sharp focus on immunologically relevant research questions.

Publication policy. Communicating the outcome of large-scale consortia-driven projects is extremely important. The research community is familiar with manuscripts that are co-authored by large numbers of investigators; however, authorship requires further consideration when multiple parties have contributed equally. Discussions with publishers indicate that assigning authorship collectively to a consortium is generally acceptable; however, for operational and pragmatic reasons, either one or a few lead investigators can be designated as named and/or corresponding authors. To appropriately acknowledge the contributions of the consortium members in general, and the work of specific investigators in particular (such as graduate students, postdoctoral researchers, statisticians and bioinformaticians), separate documents listing specific contributions can be submitted to the relevant journal as supplementary information in accordance with journal policy. In addition, this approach provides a process whereby credentials for a larger number of academic investigators can be evaluated as part of the UK government's Research Excellence Framework, a process whereby higher education institutions are allocated resources on the basis of research excellence. It is prudent for publication policies that address issues of authorship and author contributions to be defined from the outset of collaborative projects and included in the consortium agreement.

Meeting the milestones. Strategies for monitoring progress and outputs from large collaborative groups can vary from a remote approach (for example, annual

written reports), which is typical of large EU consortia, to a more intense and actively managed relationship between funder and researcher. The latter option is the chosen method for the stratified medicine consortia funded by the MRC (TABLE 1), who opted for a formal and engaging face-to-face method of review. Members of the Consortium Management Board were requested to attend face-to-face reviews of milestones and deliverables by an independent panel of experts convened by the MRC on a 6-monthly basis. Progress was robustly and critically reviewed and additional targets established or revised when required and, on occasion, suggestions for additional analyses were given. Although challenging and highly supportive, this review process was uncompromising in its expectations of milestone delivery. During each review session, the panel of experts sought to challenge the science and experimental approach of the consortium, seeking solutions at every opportunity and strategies to mitigate risk. The funding body also gained from these review sessions through a deeper understanding of the steps required to develop operational and functional research consortia.

Future directions

Using the TACERA early RA cohort, the RA-MAP Consortium set out to stratify patients with RA on the basis of clinical findings (mapping patients to distinct trajectories), whole-blood transcriptomic profiles (uncovering major disease endotypes) and clusters of serum analytes that might guide treatment choices at the time of disease onset. At the time of writing, data from the TACERA study that fulfil these aims have been submitted for publication. In the near future, the RA-MAP Consortium aims to focus on integration of these stratification tools into clinical practice. The multi-omics approach of the RA-MAP Consortium strongly indicates that disease stratification might be multi-dimensional and require stratification of patients by use of an immunological toolkit, depending on the specific clinical question being asked. Once validated, the priority will be to apply the discovered stratification algorithms in a clinical trial setting.

Conclusions

The RA-MAP Consortium, comprising more than 140 investigators, has embarked on a stimulating journey, negotiating its way through difficulties at various points along the way. The successful operation of a large consortium of academic and industry investigators relies on several key factors: the development of a functional multi-partner research infrastructure; a strong pre-competitive collaborative ethos; an uncompromising emphasis on the generation of high-quality data; the nurturing of relationships for a productive research community; the sharing of insights about understanding the disease and its treatment; and the sharing of outputs through delivery of a publication plan that targets high-impact journals. Under the existing framework of project approvals, the RA-MAP Consortium will offer the wider research community access to data and samples as soon as our own investigations have been completed. We anticipate that access to samples might be granted as early as February 2018, and to data the following year. This process will be actively managed by a dedicated Data and Sample Access Committee in a transparent manner, facilitated by a structured application form.

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1. Jones, A. & Clifford, L. Drug discovery alliances. *Nat. Rev. Drug Discov.* **4**, 807–808 (2005).
2. Barnes, M. R. *et al.* Lowering industry firewalls: pre-competitive informatics initiatives in drug discovery. *Nat. Rev. Drug Discov.* **8**, 701–708 (2009).
3. Yildirim, O., Gottwald, M., Schüler, P. & Michel, M. C. Opportunities and challenges for drug development: public-private partnerships, adaptive designs and big data. *Front. Pharmacol.* **7**, 461 (2016).
4. Schmeck, B., Bertrams, W., Lai, X. & Vera, J. Systems medicine for lung diseases: phenotypes and precision medicine in cancer, infection, and allergy. *Methods Mol. Biol.* **1386**, 119–133 (2016).
5. Stingone, J. A. *et al.* Big and disparate data: considerations for pediatric consortia. *Curr. Opin. Pediatr.* **29**, 231–239 (2017).
6. Morrison, M. "A good collaboration is based on unique contributions from each side": assessing the dynamics of collaboration in stem cell science. *Life Sci. Soc. Policy* **13**, 7 (2017).
7. Wilan, K. H. Opening up the ivory tower. *Cell* **129**, 847–850 (2007).
8. Melese, T., Lin, S. M., Chang, J. L. & Cohen, N. H. Open innovation networks between academia and industry: an imperative for breakthrough therapies. *Nat. Med.* **15**, 502–507 (2009).
9. Schleidgen, S., Klingler, C., Bertram, T., Rogowski, W. H. & Marckmann, G. What is personalized medicine: sharpening a vague term based on a systematic literature review. *BMC Med. Ethics* **14**, 55 (2013).
10. Association of the British Pharmaceutical Industry. Stratified medicine in the NHS: an assessment of the current landscape and implementation challenges for non-cancer applications. *ABPI* http://www.abpi.org.uk/our-work/library/medical-disease/Documents/stratified_med_nhs.pdf (2014).
11. Association of the British Pharmaceutical Industry. The stratification of disease for personalised medicines. Research driven recommendations to strengthen a unified UK strategy through a stakeholder alliance. *ABPI* http://www.abpi.org.uk/our-work/library/medical-disease/Documents/strat_med.pdf (2014).
12. Academy of Medical Sciences. Stratified, personalised or P4 medicine: a new direction for placing the patient at the centre of healthcare and health education. *Academy of Medical Science s* https://acmedsci.ac.uk/download?f=file&i=32644 (2015).
13. UK Trade and Investment. Unlock your global business potential. UK stratified medicine. *GOV.UK* https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/301775/UK_Stratified_Medicine.pdf (2013).

14. Medical Research Council. The Medical Research Council stratified medicine research initiatives. *MRC* https://www.mrc.ac.uk/research/initiatives/stratified-medicine/ (2017).
15. Peltonen, L. & McKusick, V. A. Genomics and medicine. Dissecting human disease in the postgenomic era. *Science* **291**, 1224–1229 (2001).
16. Loscalzo, J., Kohane, I. & Barabasi, A. L. Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. *Mol. Syst. Biol.* **3**, 124 (2007).
17. Botstein, D. & Risch, N. Discovering genotypes underlying human phenotypes: past successes for Mendelian disease, future approaches for complex disease. *Nat. Genet.* **33**, 228–237 (2003).
18. Hirschhorn, J. N. & Daly, M. J. Genome-wide association studies for common diseases and complex traits. *Nat. Rev. Genet.* **6**, 95–108 (2005).
19. Giacomini, K. M. *et al.* Genome-wide association studies of drug response and toxicity: an opportunity for genome medicine. *Nat. Rev. Drug Discov.* **16**, 70 (2017).
20. Chatterjee, N., Shi, J. & García-Closas, M. Developing and evaluating polygenic risk prediction models for stratified disease prevention. *Nat. Rev. Genet.* **17**, 392–406 (2016).
21. Berger, B., Peng, J. & Singh, M. Computational solutions for omics data. *Nat. Rev. Genet.* **14**, 333–346 (2013).
22. Hood, L., Balling, R. & Auffray, C. Revolutionizing medicine in the 21st century through systems approaches. *Biotechnol. J.* **7**, 992–1001 (2012).
23. Institute for Safe Medication Practices. The five rights: a destination without a map. *Institute for Safe Medication Practices* http://www.ismp.org/newsletters/acutecare/articles/20070125.asp (2007).
24. McInnes, I. B., Buckley, C. D. & Isaacs, J. D. Cytokines in rheumatoid arthritis — shaping the immunological landscape. *Nat. Rev. Rheumatol.* **12**, 63–68 (2016).
25. Baretta, Z., Mocellin, S., Goldin, E., Olopade, O. I. & Huo, D. Effect of BRCA germline mutations on breast cancer prognosis: a systematic review and meta-analysis. *Medicine (Baltimore)* **95**, e4975 (2016).
26. Bunting, S. F. & Nussenzweig, A. End-joining, translocations and cancer. *Nat. Rev. Cancer* **13**, 443–454 (2013).
27. Lieber, M. R. Mechanisms of human lymphoid chromosomal translocations. *Nat. Rev. Cancer* **16**, 387–398 (2016).
28. Slamon, D. J. *et al.* Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244**, 707–712 (1989).
29. Pauletti, G., Godolphin, W., Press, M. F. & Slamon, D. J. Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence *in situ* hybridization. *Oncogene* **13**, 63–72 (1996).
30. Maemondo, M. *et al.* Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N. Engl. J. Med.* **362**, 2380–2388 (2010).
31. Slamon, D. J. *et al.* Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**, 783–792 (2001).
32. Khagi, Y., Kurzrock, R. & Patel, S. P. Next generation predictive biomarkers for immune checkpoint inhibition. *Cancer Metastasis Rev.* **36**, 179–190 (2017).
33. Siniard, R. C. & Harada, S. Immunogenomics: using genomics to personalize cancer immunotherapy. *Virchows Arch.* **471**, 209–219 (2017).
34. Ramamurthy, C., Godwin, J. L. & Borghaei, H. Immune checkpoint inhibitor therapy: what line of therapy and how to choose? *Curr. Treat. Options Oncol.* **18**, 33 (2017).
35. Medical Research Council. MRC Strategic Review of Human Immunology. *MRC* https://www.mrc.ac.uk/publications/browse/strategic-review-of-human-immunology/ (2007).
36. Gerlag, D. *et al.* EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann. Rheum. Dis.* **71**, 638–641 (2012).
37. Innovative Medicines Initiative. The IMI funding model. *IMI* http://www.imi.europa.eu/about-imi/imi-funding-model (2017).
38. Johnson, W. E., Rabinovic, A. & Li, C. Adjusting batch effects in microarray expression data using Empirical Bayes methods. *Biostatistics* **8**, 118–127 (2007).
39. Herzinger, S. *et al.* SmartR: an open-source platform for interactive visual analytics for translational research data. *Bioinformatics* **33**, 2229–2231 (2017).

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Author contributions
A.P.C., M.R.B. and J.D.I. wrote the manuscript. A.P.C., S.B., F.B.-C. and A.W.P. researched data for the article. A.P.C., M.R.B., A.B., M.B., S.B., F.B.-C., B.A.F., C.S.G., P.E., M.E.R., N.G., R.H., S.H., M.F.M., I.B.M., S.R., A.W.P., F.P., D.P., R.R., A.R., M.A.S., D.S., B.T. and J.D.I. provided substantial contributions to discussions of content. A.P.C., M.R.B., A.B., S.B., F.B.-C., C.C., B.A.F., C.S.G., M.E.R., N.G., R.H., S.H., S.K., M.L., C.L., M.F.M., I.B.M., C.M.M., G.P., S.R., A.W.P., F.P., D.P., A.R., P.S.-K., M.A.S., D.S., P.C.T., B.T., W.T., D.V. and J.D.I. reviewed and/or edited the manuscript before submission.

Competing interests statement
A.P.C. declares that he has acted as a consultant for or received honoraria from BMS, Eisai, GSK, Janssen and Roche. For a full list of competing interests for all co-authors, see Supplementary information S6 (table).

Publisher's note

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SUPPLEMENTARY INFORMATION
See online article: S1 (figure) | S2 (figure) | S3 (figure) | S4 (figure) | S5 (figure) | S6 (table) | S7 (box)

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CORRIGENDUM

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

Carl Orr, Elsa Vieira-Sousa, David L. Boyle, Maya H. Buch, Christopher D. Buckley, Juan D. Cañete, Anca I. Catrina, Ernest H. S. Choy, Paul Emery, Ursula Fearon, Andrew Filer, Danielle Gerlag, Frances Humby, John D. Isaacs, Søren A. Just, Bernard R. Lauwerys, Benoit Le Goff, Antonio Manzo, Trudy McGarry, Iain B. McInnes, Aurélie Najm, Constantino Pitzalis, Arthur Pratt, Malcolm Smith, Paul P. Tak, Sander W. Tas, Rogier Thurlings, João E. Fonseca and Douglas J. Veale

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In the version of this article originally published, the author Sander W. Tas was erroneously omitted from the author list. This error has now been corrected in the online version of the article.