plate chondrocytes, which differentiate from proliferative to hypertrophic chondrocytes[6,7]. The observed abnormalities are dependent on the stage and target of the genetic recombination during the differentiation and growth process. In the context of OA, postnatally induced cartilage-specific deletion of *RBPJ* using a type II collagen-driven tamoxifen-dependent Cre system protected the joints of mice against surgically induced OA in one set of experiments[7], whereas a similar setup using either a type II collagen-driven or an aggrecan-promotor-driven tamoxifen-dependent Cre system led to the spontaneous development of OA in mice[8].These strikingly different results remain unexplained[8–10]. By targeting *Notch2* specifically, the current study confers a level of specificity that is absent in the *RBPJ*-knockout animals[4]. Loss of *RBPJ* blocks downstream signals of all Notch receptors and could also have additional effects on transcription. In the absence of the NICD–MAML–RBPJ interaction, RBPJ probably acts as a transcriptional suppressor, and its deletion might therefore have effects that are not directly related to the activation of Notch signalling[3].

Gain-of-function approaches with the other Notch receptors might provide further insights into the role of this signalling cascade in OA. In addition, several other questions remain to be addressed (Box 1). The specific interaction of ligands and receptors in Notch signalling is considered to be dependent on cell–cell contact, a feature that is not obvious in the context of the articular cartilage in which the cells are dispersed in their self-produced pericellular and intercellular matrix. Alternative modes of action could include the presence of cell extensions (filopodia) that are responsible for the cell–cell contact, or the use of exosomes within the cartilage. In addition, some evidence suggests that the ligand–receptor interaction of the Notch cascade is not limited to cell–cell interactions but can also take place within the cell[3]. The latter phenomenon would be of specific interest in articular chondrocytes. Finally, upregulation of IL-6, proposed by Zanotti et al. as a mechanism contributing to OA in *Notch2*-mutant mice, could result from an interaction between the Notch signalling cascade and other cascades such as the nuclear factor-κB signalling pathway, and might not be dependent on the NICD–MAML–RBPJ interaction[3].

In conclusion, the observation that a gain-of-function mutation in the *Notch2* gene shifts the balance of this signalling pathway towards hyper-activation and results in more severe OA in surgically challenged mice supports the view that tight regulation of cascades associated with skeletal development is essential to maintain joint homeostasis in adult life. Thus, Notch signalling seems to follow in the path of Wnt and TGFβ signalling as being an important pathway in this context[1,2].

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1. Monteagudo, S. & Lories, R. J. Cushioning the cartilage: a canonical Wnt restricting matter. *Nat. Rev. Rheumatol.* **13**, 670–681 (2017).
2. van der Kraan, P. M. The changing role of TGFbeta in healthy, ageing and osteoarthritic joints. *Nat. Rev. Rheumatol.* **13**, 155–163 (2017).
3. Siebel, C. & Lendahl, U. Notch signaling in development, tissue homeostasis, and disease. *Physiol. Rev.* **97**, 1235–1294 (2017).
4. Zanotti, S. et al. Mice harboring a Hajdu Cheney Syndrome mutation are sensitized to osteoarthritis. *Bone* **114**, 198–205 (2018).
5. Canalis, E. Clinical and experimental aspects of notch receptor signaling: Hajdu-Cheney syndrome and related disorders. *Metabolism* **80**, 48–56 (2018).
6. Kohn, A. et al. Cartilage-specific RBPjκ-dependent and -independent Notch signals regulate cartilage and bone development. *Development* **139**, 1198–1212 (2012).
7. Hosaka, Y. et al. Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development. *Proc. Natl Acad. Sci. USA* **110**, 1875–1880 (2013).
8. Liu, Z. et al. Notch signaling in postnatal joint chondrocytes, but not subchondral osteoblasts, is required for articular cartilage and joint maintenance. *Osteoarthritis Cartilage* **24**, 740–751 (2016).
9. Liu, Z. et al. A dual role for NOTCH signaling in joint cartilage maintenance and osteoarthritis. *Sci. Signal.* **8**, ra71 (2015).
10. Mirando, A. J. et al. RBP-Jκ-dependent Notch signaling is required for murine articular cartilage and joint maintenance. *Arthritis Rheum.* **65**, 2623–2633 (2013).

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SYSTEMIC SCLEROSIS

Targeting the TLR4–MD2 axis in systemic sclerosis

Steven O'Reilly and Jacob M. van Laar

Inappropriate activation of Toll-like receptor 4 (TLR4) on resident fibroblasts, through the binding of damage-associated molecular patterns, is a potential driver of fibrosis in systemic sclerosis. New evidence suggests that targeting fibroblast-specific TLR4 or an accessory molecule MD2 could have therapeutic value.

Refers to Bhattacharyya, S. et al. TLR4-dependent fibroblast activation drives persistent organ fibrosis in skin and lung. *JCI Insight* **3**, e98850 (2018).

Systemic sclerosis (SSc) is an autoimmune idiopathic connective tissue disease characterized by chronic inflammation, vascular defects and fibrosis. Among all the autoimmune rheumatic diseases, SSc has the highest all-cause mortality[1]. Persistent fibrosis is a hallmark of the disease and is caused by excessive production and accumulation of collagens and other extracellular matrix (ECM) molecules, such as fibronectin, by activated fibroblasts. Inappropriate activation of Toll-like receptors (TLRs) is associated with fibrosis in SSc and increased numbers of TLR molecules are present in patients with SSc[2,3]. Findings from a new study by Bhattacharyya et al.[4] have added considerably to our understanding of the role of TLR4 in the disease and how to target this receptor.

TLR4 is expressed on fibroblasts and seems to be important in the pathogenesis of SSc. Although lipopolysaccharide (LPS) was the first ligand described for TLR4, this receptor has since been shown to bind to other, highly diverse molecules that seem to have no structural similarities to LPS. These molecules are so-called 'danger signals' or damage-associated molecular patterns (DAMPs) and are endogenous molecules that bind to TLR4 and instigate signalling that culminates in inflammation via the activation of distinct inflammatory genes. Levels of these endogenous molecules are increased in SSc and these DAMPs seem to bind to TLR4 and promote

fibrosis (FIG. 1). Evidence for the role of TLR4 in SSc comes from the fact that *Tlr4*-global knockout mice develop less severe experimental fibrosis than wild-type controls[5] and the fact that mice lacking the gene *Tnc*, which encodes the TLR4 ligand tenascin C, are also protected from chronic fibrosis[6]. However, pharmacological targeting of TLR4 has not yet been fully realized.

Molecular profiling of SSc skin biopsy samples has revealed at least four distinct molecular subsets of SSc: an 'inflammatory intrinsic subset', a 'fibroproliferative' subset, a 'limited' subset and a 'normal-like' subset, in which the skin resembles that of healthy individuals[7]. In their study, Bhattacharyya et al.[4] first interrogated data from a meta-analysis of skin biopsy samples from 80 patients with SSc (70 with diffuse cutaneous SSc and 10 with limited cutaneous SSc) for these four distinct SSc subsets. To identify TLR4-responsive genes, they characterized the set of genes that are upregulated following ectopic expression of constitutively active TLR4 in primary skin fibroblasts (referred to as the TLR4 gene signature); the results of gene expression microarrays revealed a set of 332 upregulated genes that could be mapped to the gene ontology terms cytokines, cell migration, wound healing and ECM organization. Compared with the other SSc subsets, skin biopsy samples from patients with the inflammatory intrinsic subset of SSc were enriched for this TLR4 gene signature[4]. The authors also demonstrated that MD2 was increased in the 'inflammatory' cohort compared with other cohorts and that TLR4 and MD2 expression correlated with each other[4]. MD2 is an accessory protein that forms a TLR4–MD2 complex that is indispensable for LPS-mediated signalling[8]. Prompted by these findings and knowing that TLR4 seems to be important in SSc, the authors set out to find inhibitors for MD2.

Using in silico screening, Bhattacharyya et al.[4] identified a new inhibitor of TLR4: a β-amino alcohol derivative called T5342126. This molecule is predicted to compete with MD2 for binding to TLR4 and thus to prevent the formation of a competent TLR4 signalling complex. Initial testing of T5342126 on in vitro cultures of healthy skin fibroblasts revealed that the compound had minimal cellular toxicity and blocked in vitro inflammation mediated by ultra-pure LPS (as measured by levels of pro-inflammatory cytokines); importantly the blocking of pro-inflammatory cytokines by T5342126 was not seen when the in vitro cultures were stimulated with a specific TLR2 agonist that does not require MD2 for downstream signalling[4], suggesting that T5342126 is specific for MD2.

In a bleomycin-induced model of fibrosis (a standard inflammation-driven model of skin fibrosis), T5342126 reduced skin fibrosis by 50%, which was accompanied by a reduction in the number of myofibroblasts in the skin[4]. Treatment with the MD2 inhibitor also reduced collagen and IL-6 gene expression compared with bleomycin treatment alone. In animals with established fibrosis, treatment with T5342126 reduced dermal thickness and collagen gene expression, indicating a 'reversal' of fibrosis in an established fibrosis model[4]. This important finding suggests that targeting the TLR4–MD2 axis might have therapeutic value in SSc.

Importantly, Bhattacharyya et al.[4] showed that the MD2 inhibitor reduced the induction of collagen expression in isolated human dermal fibroblasts cultured with tenascin C or fibronectin containing extra domain A5, DAMPs that are critical in the differentiation of fibroblasts to myofibroblasts[6,9] and that are implicated in SSc pathogenesis. These DAMPs are particularly intriguing in the context of fibrosis as they are not normally expressed in the adult body during homeostasis. For instance, expression of tenascin C is restricted postnatally, and it is only expressed upon the occurrence of tissue damage. This observation suggests that when tissue damage occurs in SSc, increased secretion of tenascin C helps to repair the damage. Failure to terminate this wound-healing response is speculated to underpin the disease. In other words, a programmed wound healing response that is sensed by TLR4 and mediated through DAMPs is beneficial, but failure to terminate this response leads to SSc. Whether the novel MD2 inhibitor tested here can diminish signalling by other DAMPs remains an open question.

> "targeting the TLR4–MD2 axis might have therapeutic value in SSc"

Previously, monocytes and/or macrophages were thought to be the predominant cell type(s) mediating pro-fibrotic effects through TLR4 signalling[9]. Given that only global *Tlr4*-knockout mice have previously been used, it has been difficult to delineate the precise role of monocytes and macrophages as opposed to resident fibroblasts in this context. The finding that specific genetic ablation of TLR4 in fibroblasts resulted in attenuated fibrosis[4] suggests that fibroblasts are the primary cell type responsible for TLR4-mediated fibrosis.

Interestingly, the small-molecule TLR4 antagonist eritoran failed to demonstrate efficacy in a phase III trial for severe sepsis[10], perhaps owing to the biological complexity of sepsis (that is, targeting one specific pathway is insufficient, owing to redundancy). However, using a small-molecule inhibitor of MD2 might yield clinical benefits in SSc owing to the unique reliance of TLR4-mediated fibroblast activation on MD2, regardless of the ligand used, be it LPS or a DAMP (FIG. 1). Furthermore, the use of a specific MD2–TLR4 inhibitor that targets a protein–protein interaction to abolish TLR-mediated fibrosis is a unique approach, as typically, protein–protein interactions are considered difficult drug targets owing to the difficulty of inhibiting multiple interactions with small molecular weight drugs (<500 Da).

Given the fact that the novel MD2 inhibitor identified by Bhattacharyya et al.[4] not only blocked but reversed fibrosis in relevant animal models, and the association with the TLR4 gene signature in a specific subset of patients (the inflammatory intrinsic subset), it would seem that targeting TLR4 signalling pathways in these patients could result in the greatest clinical benefit. Disease heterogeneity is notorious in SSc and identifying the appropriate patients for precision medicine approaches seems likely to hold great promise. A strategy such as this, in which patients with SSc can be profiled at the molecular level to guide physicians towards the appropriate treatment, is a welcome addition. Moreover, further lead optimization of the MD2 inhibitor T5342126 might improve its efficacy.

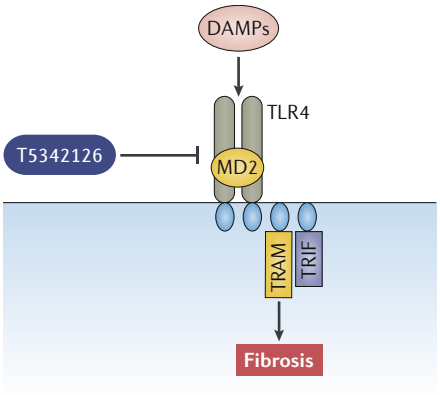

Fig. 1 | **Targeting TLR4–MD2 signalling in fibroblast activation.** Damage-associated molecular patterns (DAMPs) such as tenascin C signal via Toll-like receptor 4 (TLR4), which requires the accessory protein MD2 for signalling to occur, thereby activating various downstream signalling molecules that ultimately lead to the differentiation of fibroblasts to myofibroblasts and to fibrosis. Inhibiting MD2 using T5342126 attenuated fibrosis mediated by TLR4 stimulation. TRAM, TRIF-related adapter molecule; TRIF, TIR domain-containing adapter protein inducing interferon-β.

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https://doi.org/10.1038/s41584-018-0077-6

1. Elhai, M. et al. Trends in mortality in patients with systemic sclerosis over 40 years: a systematic review and meta-analysis of cohort studies. *Rheumatology* **51**, 1017–1026 (2012).
2. O'Reilly, S. Toll like receptors in systemic sclerosis: an emerging target. *Immunol. Lett.* **195**, 2–8 (2018).
3. Fullard, N. & O'Reilly, S. Role of innate immune system in systemic sclerosis. *Sem. Immunopathol.* **37**, 511–517 (2015).
4. Bhattacharyya, S. et al. TLR4-dependent fibroblast activation drives persistent organ fibrosis in skin and lung. *JCI Insight* **3**, e98850 (2018).
5. Bhattacharyya, S. et al. Toll-like receptor 4 signaling augments transforming growth factor-β responses: a novel mechanism for maintaining and amplifying fibrosis in scleroderma. *Am. J. Pathol.* **182**, 192–205 (2013).
6. Bhattacharyya, S. et al. Tenascin-C drives persistence of organ fibrosis. *Nat. Commun.* **7**, 11703 (2016).
7. Milano, A. et al. Molecular subsets in the gene expression signatures of scleroderma skin. *PLoS ONE* **3**, e2696 (2008).
8. Park, B. S. et al. The structural basis of lipopolysaccharide recognition by the TLR4–MD-2 complex. *Nature* **458**, 1191 (2009).
9. Dowson, C. et al. Innate immunity in systemic sclerosis. *Curr. Rheumatol. Rep.* **19**, 2 (2017).
10. Opal, S. M. et al. Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: The ACCESS randomized trial. *JAMA* **309**, 1154–1162 (2013).

Competing interests
The authors declare no competing interests.

RHEUMATOID ARTHRITIS

Unravelling how glucocorticoids work in rheumatoid arthritis

Rowan Hardy and Mark S. Cooper

Using mice with targeted deletion of the glucocorticoid receptor, a new study has examined the cell types that mediate the anti-arthritic effects of therapeutic glucocorticoids. Surprisingly, in the serum transfer-induced arthritis model, glucocorticoids target stromal cells rather than immune cells.

Refers to Koenen, M. et al. Glucocorticoid receptor in stromal cells is essential for glucocorticoid-mediated suppression of inflammation in arthritis. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2017-212762 (2018).

Despite being available for almost 70 years, we are only now beginning to understand the mechanism of action of glucocorticoids, a class of drugs that are essential in the treatment of rheumatoid arthritis (RA). In a new article, Koenen et al.[1] use a range of elegant in vivo approaches to investigate how glucocorticoids reduce joint inflammation in the serum transfer-induced arthritis (STIA) model. Bone marrow chimaeras (generated by irradiating mice to destroy immune cells and then using donor cells to repopulate the marrow) and inducible genetic deletion of *NR3C1* (which encodes the glucocorticoid receptor) using a tamoxifen-inducible Cre–*lox* system enabled mice to be generated with selective deletion of the glucocorticoid receptor in either the haematopoietic or stromal compartment. Absence of the glucocorticoid receptor in haematopoietic cells had no effect on the ability of dexamethasone to suppress joint swelling and inflammation. However, absence of the glucocorticoid receptor in the stromal compartment rendered mice unresponsive to the effects of the glucocorticoid dexamethasone on joint inflammation.

This work follows on from the study of an alternative mouse model of joint inflammation termed adjuvant-induced arthritis (AIA)[2]. Interestingly, in AIA mice the anti-inflammatory effects of glucocorticoids were dependent on the presence of the glucocorticoid receptor in T cells, in which type 17 responses seemed to be major targets. In this model, there seemed to be no contribution from glucocorticoid receptors present in the stroma. The situation is further complicated by findings from other inflammatory models, which suggest that glucocorticoid receptors within macrophages are crucial to the mechanism of action of glucocorticoids[3]. These findings highlight the fact that although glucocorticoids have similar therapeutic effects in different inflammatory models, these effects might be mediated by different cell types. An unresolved question is how each of these mouse models translates into the clinical situation of RA and related joint diseases.

Although not traditionally thought of as a major target of glucocorticoids, the stroma is now implicated as a target of glucocorticoid action in inflammatory arthritis. Mice with

> “the stroma is now implicated as a target of glucocorticoid action in inflammatory arthritis”

deletion of the glucocorticoid receptors in chondrocytes have exaggerated inflammation in both the AIA and STIA models, highlighting a possible anti-inflammatory role for chondrocyte glucocorticoid signalling in arthritis[4]. Somewhat paradoxically, glucocorticoid signalling in osteoblasts seems to be pro-inflammatory with blockade of the glucocorticoid receptor resulting in reduced inflammation in STIA mice[5]. In the study by Koenen et al.[1] the stromal cells seem to regulate inflammation by polarizing macrophages towards a suppressive phenotype, characterized by increased efferocytosis and expression of anti-inflammatory markers. How chondrocytes or osteoblasts affect inflammation is not clear. These results collectively indicate that several stromal cell types (synovial fibroblasts, chondrocytes and osteoblasts), in addition to immune cells, can mediate the effects of glucocorticoids on joint inflammation (FIG. 1).

An additional level of regulation of glucocorticoid action is the glucocorticoid metabolizing enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). This enzyme amplifies glucocorticoid signalling through the local activation of glucocorticoids from inactive precursors, and is critical to the conversion of inactive glucocorticoids such as cortisone and prednisone to their active counterparts, cortisol and prednisolone. Importantly, 11β-HSD1 does not metabolize dexamethasone. Mice defective in 11β-HSD1 develop exaggerated joint inflammation in acute and chronic models of arthritis[6,7]. 11β-HSD1 activity within macrophages seems to be critical in restraining inflammation in these models[8]. It would be interesting to know whether the responses detected by Koenen et al.[1] with dexamethasone are similar using glucocorticoids that are regulated by 11β-HSD1 such as those typically used to treat patients with RA (cortisone and prednisone).

The novel finding that the stroma could be an important target opens up the prospect of developing medications that have the beneficial anti-inflammatory effects of glucocorticoids without the adverse effects. Early attempts to develop more selective glucocorticoids, known as selective glucocorticoid receptor agonists (SEGRAs), revolved around the notion that the beneficial effects of glucocorticoids were a result of the monomeric glucocorticoid receptor interfering with pro-inflammatory signalling pathways (termed repression), whereas adverse effects were a