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REVIEW

Pathogenic stromal cells as therapeutic targets in joint inflammation

(NRR-17-203V4)

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Key words: stroma, inflammation, musculoskeletal, fibroblast, joint, mesenchymal

1 **Abstract**

2

3 Knowledge of how the joint functions as an integrated unit in health and disease
4 requires an understanding of the stromal cells populating the joint mesenchyme,
5 including fibroblasts, tissue resident macrophages and endothelial cells.
6 Physiological and pathological mechanisms in these mesenchymal cells that define
7 the joint have begun to cast new light on why joint inflammation persists. In this
8 review, we highlight how the shared embryological origins of fibroblasts and
9 endothelial cells may shape the behaviour of these cell types in diseased adult
10 tissues. We review the molecular mechanisms by which cells of mesenchymal origin
11 sustain inflammation in the synovial membrane and tendons, highlighting the
12 importance of recently discovered fibroblast subtypes and their associated cross talk
13 with endothelial cells, tissue resident macrophages and leukocytes. Finally, we
14 discuss how this knowledge shapes the future therapeutic landscape, emphasising
15 the requirement for new strategies to address the pathogenic stroma and associated
16 cross talk of leukocytes with cells of mesenchymal origin.

17

18 **Key points**

19

- 20 • Joint inflammation and tissue damage are mediated by stromal cells ~~derived~~
21 from of embryonic mesodermal origin
- 22 • Stromal activation and inflammation “memory” of previous inflammatory
23 insults are shared ~~disease~~ mechanisms exhibited by fibroblasts, tissue
24 resident macrophages and ~~and~~ endothelial cells
- 25 • Recent advances characterising the phenotype and function of cells of
26 mesenchymal origin highlight thee distinct fibroblast subtypes mediating joint
27 inflammation and tissue damage
- 28 • Mesenchymal stromal cell niches and their interactions with leukocytes are
29 implicated in the persistence of joint inflammation
- 30 • To be effective, strategies to treat residual joint disease should target
31 pathogenic stroma and associated immune cell cross talk

32

33

34 Introduction

35

36 Chronic inflammatory diseases affecting joint soft tissues include arthritis
37 (synovium and cartilage), enthesopathy and tendinopathy. Collectively, these
38 diseases comprise a significant global economic burden¹. Each are characterized
39 by inflammation of mesenchymal tissues that form the synovium, tendons,
40 ligaments and joint capsule and in some cases structural damage to bone and
41 cartilage. Inflammation of these tissues is broadly characterized by leuckocyte
42 infiltration, fibroblast accumulation and neovascularization supporting cell
43 expansion. In this article, we first review the pathophysiological basis of
44 inflammation and tissue damage with respect to the embryological origins of joint
45 mesenchymal tissues. We next discuss the stromal cell types populating joint
46 mesenchymal tissues, including fibroblasts, tissue resident macrophages (TRM)
47 and endothelial cells (vascular and lymphatic), highlighting their contribution and
48 roles in chronic synovial inflammation and tissue damage. Finally, we discuss
49 potential future therapeutic strategies to target inflammation across joint
50 mesenchymal tissues that address the pathogenic stroma and associated immune
51 cell cross talk.

52

53 1.0 Embryological origins of the tissues that mediate inflammation 54 and damage across the whole joint organ

55

56 Inflammation and tissue damage are pivotal pathological processes affecting tissues
57 structures across the whole joint organ. To further understand the mechanisms and
58 inter-relationships underpinning these fundamental disease processes, it is important
59 to consider the origins of joint tissues, given that an organ is best defined by its
60 embryological origin as well as function. This section discusses how the
61 embryological and anatomical origins of the tissues that comprise the joint might
62 shape inflammation and tissue damage, highlighting how this knowledge informs
63 understanding of 'disease patterns' across the joint.

64

65 The anatomical basis of inflammation and tissue damage relative to their
66 embryological origin is summarized in Figure 1. Although parts of the axial skeleton

67 | ~~derive from neural crest, Embryonic~~ mesoderm is the precursor for mesenchymal
68 | tissues comprising the ~~axial and~~ appendicular skeleton, synovium, cartilage,
69 | tendons, ligaments, joint capsule and their associated lymphatics and vasculature.
70 | These joint soft tissues are predominantly composed of cells of mesenchymal origin,
71 | including fibroblasts, vascular and lymphatic endothelial cells and TRM. The ~~shared~~
72 | embryological origins of ~~stromal fibroblasts and endothelial~~ cells may shape the
73 | behaviour of these cell types in diseased adult tissues. Notably, ~~mesoderm derived~~
74 | fibroblast and endothelial cell populations both undergo sustained phenotypic
75 | changes after exposure to inflammatory stimuli, exhibiting stromal activation and ~~a~~
76 | ~~form of tissue~~ 'memory'^{2,3}. ~~However, the distinct molecular markers expressed by~~
77 | ~~these cell types vary, as we later discuss. TRM also exhibit complex activation~~
78 | ~~states and "memory"~~⁴. The origins and renewal of TRM have been extensively
79 | reviewed ~~and will not be repeated here~~⁵⁻⁸. The majority of TRM are established
80 | during embryonic development and persist into adulthood, rather than replacement
81 | from circulating adult monocytes^{7,9-14}. During early gestation, macrophages are first
82 | observed and expand in the extraembryonic yolk sac during primitive hematopoiesis.
83 | Yolk sac derived hematopoietic stem cells (HSCs) emerge to form bone marrow
84 | precursor cells, which subsequently gives rise to all immune cell lineages^{7,15} (Figure
85 | 1). Importantly, yolk sac derived TRM are ~~phenotypically~~ distinct from HSC derived
86 | progeny¹⁰. The subspecialized adult tissue niches which TRM occupy dictate
87 | heterogeneity in the phenotype and functions of these cells in health and disease¹⁶.
88 | We next review how these mesenchymal cell populations are implicated in mediating
89 | inflammation and tissue damage in joint disease.

90

91 | **2.0 Cells of mesenchymal origin in the ~~healthy and diseased~~ joint**

92

93 | In this section, we focus exclusively on cells of mesenchymal origin including
94 | fibroblasts, endothelial cells and TRM rather than on haematopoietically derived cells
95 | whose role in these processes (in particularly inflammation and damage) has been
96 | well documented¹⁷⁻¹⁹. We discuss the roles of these cells in normal joint physiology
97 | and their impact on inflammation and damage in joint disease. We highlight the
98 | recently identified mechanisms implicated in sustaining synovial inflammation,

99 discussing the molecular features and pathological phenotypes of fibroblast
100 subtypes, endothelial cells and TRM.

101 **2.1 Fibroblasts and the healthy joint**

102

103 The term 'stroma' was originally derived from the Greek word describing "a platform
104 on which to lie" and is used to describe the supporting substance of tissue. Its
105 principle role is to maintain the microenvironment required by the parenchyma; the
106 important functional elements of each body system. The stroma comprises
107 connective tissue, nerves, vessels and the extracellular matrices (ECM) and fluids
108 which these cells produce²⁰. Joint soft tissues including synovium, capsule, tendon
109 and enthesis are ~~predominantly composed of mesenchymal stromal cells~~
110 ~~of cellular and acellular ECM~~. Fibroblasts are the most abundant cell type populating
111 these joint connective tissues²¹ and synthesise the highly organized collagen rich
112 scaffold necessary for joint structure and movement.

113

114 Fibroblasts are defined by their spindle shaped morphology, the absence of specific
115 lineage markers of leukocytes, endothelium and epithelium and their ability to adhere
116 to tissue culture plastic *in vitro*²². They are believed to arise from 3 distinct cellular
117 origins: primary mesenchyme, local epithelial-mesenchymal transition (EMT) or bone
118 marrow derived precursors (circulating fibrocytes)^{23,24}. It is widely accepted that the
119 majority of fibroblasts originate from primary mesenchymal cells and that fibroblasts
120 can proliferate to generate new progeny^{25,26}. In physiological conditions, fibroblasts
121 provide mechanical strength to tissues by producing ECM components (type I, III
122 and V collagen and fibronectin) as well as factors that regulate ECM turnover,
123 including metalloproteinases (MMPs) and proteins involved in the formation of
124 basement membranes (type IV collagen and laminin)^{27,28}. Fibroblasts synthesise an
125 array of paracrine factors²⁹ and exhibit mechanosensitive properties³⁰ to effect
126 functional adaptation in normal joint physiology. The intimate relationship between
127 fibroblasts and mesenchymal stromal cells (MSC) and the clinical use of MSC to
128 repair damaged tissues has driven a renewed interest in fibroblasts as new
129 therapeutic targets²¹.

130

131 **2.2 Mechanisms sustaining joint inflammation based on pathogenic stroma**

132

133 **2.2.1 Fibroblasts and the diseased joint**

134

135 Traditionally, the diversity of stromal cells and in particular fibroblasts and their roles
136 beyond those of space filling and ECM homeostasis have been underexplored in
137 inflammation. Mesenchymal tissues in the joint including the synovium, ~~capsule~~,
138 entheses and tendons undergo phenotypic changes as a consequence of
139 inflammation³¹⁻³³. These include molecular and structural changes to the ECM,
140 impacting upon the functional quality of the healed tissue³⁴. Whilst it remains
141 challenging to discern which is the initiating pathogenic cell type, it is clear that
142 stromal cells populating these tissues provide a niche conducive to sustaining
143 chronic inflammation^{2,35,36}. Recent work shows that fibroblasts vary phenotypically
144 and functionally at different anatomical sites and contribute significantly to the
145 identity of individual tissues, providing a so-called 'stromal postcode'²⁶. Furthermore,
146 it is known that, rather than acting as a bystander, fibroblasts are capable of actively
147 participating and indeed orchestrating inflammation and immunity³⁶⁻³⁸. We next
148 review how fibroblasts sustain inflammation, highlighting the mechanisms
149 underpinning their activation, "memory" and phenotypic diversity, with particular
150 focus on the synovial microenvironment.

151

152 ***Fibroblast activation and memory***

153

154 Fibroblast activation is a recognized feature of diseases affecting the joint, whereby
155 fibroblasts adopt a pro-inflammatory phenotype. This pathological feature has been
156 identified in cancer³⁹, rheumatoid synovium^{32,33} and tendon disease³¹. Fibroblast
157 activation and memory therefore span both innate and adaptive immune responses,
158 suggesting this is a highly conserved disease mechanism common to tissues of
159 mesenchymal origin. There is now a growing list of cell surface molecules and
160 secreted products which collectively provide a fibroblast activation marker "cassette".
161 These include CD90 (Thy1), CD44, decay accelerating factor (CD55), VCAM-1
162 (CD106), uridine diphosphoglucose dehydrogenase, and prolyl-4-hydroxylase,
163 Podoplanin (PDPN/gp38), endosialin (CD248) and Fibroblast Activation Protein
164 (FAP)^{31,36,37,40-42}. Fibroblast activation markers therefore represent important
165 phenotypic alterations implicated in effecting the switch from resolving to persistent
166 inflammation⁴².

167
168 Epigenetic changes are implicated in fibroblast activation and memory. New insights
169 into the epigenetics of inflammatory rheumatic diseases have been recently
170 reviewed in detail elsewhere ⁴³. Prolonged exposure of RA synovial fibroblasts to
171 TNF α reduce histone H4 levels and promote H4 acetylation ⁴⁴. This study showed
172 that TNF α removed the chromatin barrier from the CXCL10 promoter, permitting
173 abundant binding of NF- κ B family transcription factors and recruitment of
174 transcriptional machinery ⁴⁴. DNA methylation is another important epigenetic
175 modification identified in RA synovial fibroblasts occurring during the early stage of
176 disease ⁴⁵. Further studies are required to identify the mechanisms underpinning
177 DNA methylation and there appears to be important prognostic potential for
178 differentially methylated genes as disease biomarkers ⁴⁵. The activated and
179 aggressive phenotype of RA synovial fibroblasts is associated with global DNA
180 hypomethylation ⁴⁶. Gaur *et al.* investigated if microRNAs moderate the methylation
181 status of RA synovial fibroblasts, showing L-methionine increased DNA methylation
182 compared to betaine ⁴⁷. Collectively these studies advance our understanding of how
183 epigenetic changes are implicated in fibroblast activation and memory, informing
184 future strategies to selectively target pathogenic fibroblasts.

185 ~~Recent work shows that tissue resident fibroblasts help define the pattern of joints~~
186 ~~involved, not only in arthritis but in other diseases with a prominent stromal~~
187 ~~component~~ ³⁹.

188 ~~Importantly, this concept of epigenetically-driven anatomical diversity of synovial~~
189 ~~fibroblasts provides an attractive mechanism to explain the clinical observations that~~
190 ~~different types of arthritis affect distinct types of joints. For example, OA and PsA~~
191 ~~often involve the distal interphalangeal joints, whereas RA is frequently symmetrical~~
192 ~~and more commonly affects the MCP joints. In contrast, AS mainly targets spinal~~
193 ~~ligaments and enthesal tissue~~ ⁴⁰. Such studies have prompted improved
194 ~~characterization of the phenotypes of fibroblast subsets and their different proposed~~
195 ~~roles. In RA, synovial fibroblasts undergo distinct changes in function, including loss~~
196 ~~of immunosuppressive response in early disease, followed by later acquisition of an~~
197 ~~immuno-stimulatory phenotype~~ ⁴⁴.

198

199 | Fibroblasts from different joint tissues maintain their phenotype, positional memory
200 | and topographic differentiation despite culture *ex vivo*. Fibroblasts isolated from RA
201 | synovium or diseased tendon exhibit stromal 'memory', whereby these cells show an
202 | enhanced subsequent capacity to respond to an additional inflammatory stimulus
203 | ^{2,31,44}. Therefore, sustained expression of activation markers by fibroblasts in the joint
204 | reflects their 'primed' status after exposure to an inflammatory stimulus. ~~In addition to
205 | fibroblast activation, this concept of stromal memory also spans innate and adaptive
206 | immunity, suggestive of a highly conserved disease mechanism across tissues of
207 | mesenchymal origin.~~ The processes underpinning innate memory have been
208 | extensively reported for leukocytes ^{48,49} and are gaining acceptance in tissue
209 | resident cells of mesenchymal origin. Engagement of TLR4 and downstream
210 | activation of the NFκB pathway is a prominent pathological feature of fibroblasts
211 | populating inflamed joint tissues ^{2,31,44}. These studies suggest that fibroblast memory
212 | is associated with altered NFκB responsiveness to an inflammatory stimulus ⁵⁰.
213 | Given the longevity of fibroblasts as tissue resident cells and the relatively low rates
214 | of tissue cell turnover in the joint ⁵¹, the effects of stromal memory in tissues such as
215 | synovium and tendon are likely to be long lived. In contrast, dermal fibroblasts show
216 | higher rates of turnover and do not exhibit ~~stromal~~ memory, suggesting this ~~disease~~
217 | ~~mechanism~~process of stromal memory may vary according to anatomical location
218 | ^{2,52,53}. ~~Rheumatic diseases follow a characteristic anatomical pattern of joint and
219 | organ involvement. Mechanisms regulating the predilection of specific joints for
220 | developing particular forms of arthritis (for example osteoarthritis (OA) compared to
221 | rheumatoid arthritis (RA)) have been reviewed in detail ⁵⁴. These include site-specific
222 | local cell types driving disease, systemic triggers affecting local cell types and site-
223 | specific exogenous factors activating cells locally. Therefore the mechanisms
224 | underpinning activation of stromal cells depends on the local anatomical tissue
225 | niche ⁵⁴.~~

227 **Fibroblast diversity**

228 | ~~Recent work shows that tissue resident fibroblasts help define the pattern of joints
229 | involved in RA ^{55,56}. The concept of epigenetically-driven anatomical diversity of
230 | synovial fibroblasts provides an attractive mechanism to explain the clinical
231 | observations that different types of arthritis affect distinct types of joints. For~~

232 example, OA and PsA psoriatic arthritis often involve the distal interphalangeal joints,
233 whereas RA is frequently symmetrical and more commonly affects the MCP joints. In
234 contrast, AS ankylosing spondylitis (AS) mainly targets spinal ligaments and
235 enthesal tissue⁵⁷. Such studies have prompted improved characterization of the
236 phenotypes of fibroblast subsets and their different proposed roles. In RA, synovial
237 fibroblasts undergo distinct changes in function, including loss of
238 immunosuppressive response in early disease, followed by later acquisition of an
239 immuno-stimulatory phenotype⁵⁸. ~~Fibroblasts show considerable variability according~~
240 ~~to genetic and hormonal factors between individuals~~. Highly conserved homeobox
241 (HOX) transcription factors specify regional identities of cells and tissues
242 throughout the body^{59,60} and adult fibroblasts retain key features of embryonic
243 positional HOX gene expression⁵⁶. Fibroblasts also vary according to their
244 anatomical location in relation to tissue structures at an individual site and the
245 exogenous stimuli which they receive^{54,56,61}. Whether variability can be attributed to
246 the plasticity of individual fibroblasts necessary for responding to different
247 environmental cues and whether phenotypic variation can be used to define distinct
248 subsets of fibroblasts specialized for different niches remains unclear.

249
250 The synovium is composed of lining and sub-lining layers of fibroblasts which vary in
251 terms of phenotype and function ~~according to their anatomical sub-location~~. Single
252 cell RNA sequencing and immunohistochemistry have revealed that RA synovial
253 fibroblasts can be broadly characterized into 3 subsets, highlighted in Figure 2.
254 Synovial lining fibroblasts are CD34⁻CD90⁻CD55⁺ and Cadherin 11⁺. This lining
255 subset synthesizes MMP-1 and MMP-3 which mediate tissue damage in the
256 inflamed joint⁶². Fibroblasts populating the synovial sublining are predominantly
257 comprised of 2 populations. CD34⁺CD90⁻ fibroblasts release CXCL12, CCL2 and IL-
258 6 and ~~mediate-drive fibroblast accumulation cell-proliferation~~ and invasion. A second
259 population of CD34⁻CD90⁺ fibroblasts with a pro-inflammatory phenotype highly
260 express markers of ~~stromal~~ fibroblast activation^{62,63}. These ~~'pathogenic'~~ fibroblast
261 subsets between them degrade articular cartilage, mediate stromal memory, sense
262 tissue damage via TLR4 activation and have altered responsiveness to signalling
263 pathways converging on NFκB responsiveness^{26,33,50,62} (Figure 2). Having
264 highlighted the complexity of discrete synovial fibroblast subtypes, we next discuss

Comment [MOU1]: Chris can you suggest a reference for this statement?

265 the phenotypes and functions of other mesenchymal cell types including endothelial
266 cells and TRM and their respective roles in joint ~~health and~~ disease.

267

268 **2.2.2 The endolymphatic niche in the ~~healthy and~~ diseased joint**

269

270 Other mesenchymal stromal tissues including the vasculature and lymphatics
271 contribute to sustaining inflammation across the joint organ. Neo-angiogenesis is a
272 prominent feature of disease of mesenchymal joint tissues and impacts upon
273 changes in tissue architecture and pain perception⁶⁴. In health, vascular endothelial
274 cells regulate blood flow, vessel wall permeability and leukocyte extravasation into
275 tissues, regulating the inflammatory process⁶⁵⁻⁶⁸. In lymph nodes and tertiary
276 lymphoid tissues, high endothelial vessels (HEVs) provide specialized
277 microenvironments for efficient entry of lymphocytes into tissues in an L-selectin
278 dependent process⁶⁹. The phenotypes of endothelial cells change as inflammation
279 transitions from acute to chronic and also between activation of innate and adaptive
280 immune systems⁶⁷. Endothelial cell phenotypes are poorly characterized in tendon
281 and enthesal tissues. However, in RA synovium, these cells have been described
282 as activated, angiogenic, apoptotic and leaky, a process found in many tumour
283 microenvironments⁷⁰. During prolonged exposure to inflammatory stimuli endothelial
284 cells become activated, exhibit memory and express adhesion molecules including
285 ICAM, VCAM-1 and CD31 (PECAM-1)^{3,71-73} (Figure 2). These activated endothelial
286 cells ~~subsequently also~~ present chemokines and initiate leukocyte migration from
287 blood to local tissues⁷⁰. Endothelial activation is a cause and consequence of
288 endothelial dysfunction^{74,75}, culminating in increased microvascular permeability,
289 extravasation of plasma and joint oedema. Release of angiogenic factors including
290 VEGF triggers angiogenesis, provide necessary nutrients and oxygen to meet the
291 metabolic demands of the inflamed tissue. Importantly, neo-angiogenesis further
292 promotes the retention and survival of immune cells at inflamed sites, thereby
293 sustaining chronic inflammation³⁸. These angiogenic processes occur during normal
294 inflammatory immune responses (i.e vaccination)⁷⁶, however whether angiogenesis
295 that occurs in joint disease is a cause or effect of pathology remains unclear.

296

297 Stromal lymphatic vessels form a one-way conduit for tissue fluid and leukocytes in
298 health and disease⁷⁷. During adaptive immune responses, antigen presenting cells

299 travel to lymph nodes via lymphatic vessels, which highly express PDPN, implicated
300 in ~~stromal~~ fibroblast activation⁷⁸. The permeability of lymphatic vessels is a tightly
301 regulated dynamic process that alters during health and disease⁷⁹. Lymphatic
302 vessel growth (lymphangiogenesis) is a primary response during acute inflammation,
303 which becomes dysregulated in chronically inflamed adult tissues⁸⁰. In experimental
304 murine models of inflammatory arthritis, lymphatic vessels and nodes draining the
305 diseased joint undergo an initial expansion phase to expedite lymphatic clearance.
306 This expansion phase is followed by a collapsed phase, characterized by structural
307 damage to lymphatic vessels and reduced lymphatic clearance^{79,81}. Studies
308 demonstrate alteration in lymphatic vessel function and lymph node volume also
309 occur in patients with RA flare⁸². Therapies targeting aberrant lymphatic function
310 have shown promise in preclinical models of inflammatory arthritis and may prove
311 efficacious in RA⁷⁹.

312

313 **2.2.3 Tissue Resident Macrophages in the ~~healthy and~~ diseased joint**

314

315 TRM mediate a diverse range of biological actions. They are appropriately positioned
316 and transcriptionally primed to respond to local environmental challenges,
317 maintaining tissue homeostasis. TRM direct immune surveillance, induce
318 inflammation and promote subsequent resolution, ~~reviewed in detail elsewhere~~^{34,83}.
319 Given the biological complexity of these roles, TRM are highly heterogeneous and
320 exhibit diverse phenotypic and functionally distinct subtypes within a single tissue
321 type^{5,84}.

322

323 In inflamed synovium, TRM mediate immune surveillance through expression of a
324 variety of ~~pattern recognition receptors~~DAMPs, notably ~~Toll-like receptors (TLR)~~
325 TLR2 and TLR4 and facilitate the recruitment of infiltrating leukocytes, including
326 monocyte derived macrophages⁸⁵⁻⁸⁷. TRM induce joint inflammation through release
327 of TNF α , IL-1 β IL-6, GM-CSF and PGE₂, driving fibroblast ~~accumulation~~proliferation,
328 angiogenesis, leukocyte recruitment and tissue damage via protease secretion
329 (Figure 2). The essential role of non-classical Ly6C-monocytes has been reported in
330 murine arthritis models⁸⁸. This study highlights the phenotypic heterogeneity of
331 synovial TRM, demonstrating how macrophage activation status regulates disease

332 progression and resolution. In support of this, human RA synovial macrophages
333 exhibit distinct transcriptional profiles associated with disease activity and therapy ⁸⁹.
334 However, distinction between TRM and infiltrating macrophages is currently
335 hampered by a lack of specific markers that distinguish between these populations in
336 diseased human tissues.

337

338 | The pro-inflammatory milieu in the inflamed synovium triggers an active process of
339 lipid mediator class switching and the subsequent release of families of specialized
340 proresolving mediators (SPM). These include lipoxins, resolvins, protectins and
341 maresins, that are generated via transcellular biosynthesis and are concerned with
342 mediating resolution of inflammation⁹⁰⁻⁹⁴. These bioactive lipid mediators initiate
343 programmes which halt neutrophil infiltration, potentiate monocyte recruitment,
344 moderate vascular permeability and promote phagocytosis and drainage of apoptotic
345 cells⁹⁵. The mechanisms mediating resolution in inflammatory arthritis have been
346 reviewed in detail and are not covered here ~~are reviewed in detail elsewhere~~⁹⁶. TRM
347 are key regulators of repair and fibrosis across all tissue types ³⁴ and are also
348 implicated in mediating resolution of inflammation. Distinct populations of resolution
349 phase macrophages have been identified in systemic murine inflammation models
350 that express Alox15, Timd4 and Tgfb2, which terminate leukocyte recruitment and
351 promote clearance ⁹⁷. However, the precise phenotypes of TRM mediating effecting
352 resolution in human joint disease requires further investigation.

353

354

355

356 **2.2.4 Cross talk between cells of mesenchymal origin**

357

358 Having highlighted the molecular features and phenotypes of mesenchymal cells
359 and their roles in mediating joint pathology, we next discuss how cross talk between
360 these cell populations sustains inflammation. Damage sensing mechanisms,
361 cytokine and chemokine gradients are pivotal pathological processes involving cross
362 talk between fibroblast, endothelial cell, TRM and leukocyte populations that sustain
363 inflammation in the diseased joint ^{26,98,99}.

364

365 RA synovial fibroblasts act as sentinel cells that can “sense” tissue damage. This
366 occurs via the binding of damage associated molecular patterns (DAMPs) including
367 HMGB1, heat shock and S100 proteins^{100,101}. [Tenascin-C a matrix protein induced](#)
368 [upon tissue damage also activates TLR4 mediated sterile inflammation](#)¹⁰². Binding
369 of these ligands to TLR4 induces a high alert state, favouring the development of
370 chronic inflammation^{50,103}. Engagement of TLR4 activates Myd88 signalling
371 pathways, inducing pro-inflammatory cytokine release via NFκB activation⁴⁸.
372 Consequently, activated synovial fibroblasts are primed to release a broad range of
373 pro-inflammatory mediators. These localised cytokine and chemokine gradients
374 promote the migration, retention and survival of leukocytes and TRM,^{42,104} creating a
375 complex functional syncytium conducive to sustaining inflammation, highlighted in
376 Figure 3. The processes mediating leukocyte trafficking between stromal
377 compartments in RA are recently reviewed in detail elsewhere¹⁰⁵.

378

379 *Fibroblast – immune cell cross talk*

380

381 RA synovial fibroblasts promote leukocyte retention via release of cytokines and
382 chemokines and via contact with other cells of mesenchymal origin. Pro-
383 inflammatory cytokines released by retained monocytes, T cells and TRM including
384 IFN γ , TNF α and IL-1 β induce activated synovial fibroblasts to release high levels of
385 PGE₂, GM-CSF, IL-6. These cytokines exert differing effects on leukocyte activation.
386 PGE₂ moderates chemokine production and promotes Th2, Th17 and Treg
387 responses¹⁰⁶. IL-6 drives CD4+ T cells towards Th17 activation¹⁰⁷, whereas GM-
388 CSF promotes neutrophil survival and monocyte differentiation in the inflamed
389 synovium^{26,108}. [Nguyen et al. demonstrated that IL-6 and other inflammatory](#)
390 [cytokines and chemokines are regulated by a positive feedback loop that selectively](#)
391 [operates in fibroblasts involving leukemia inhibitory factor \(LIF\), LIF receptor and](#)
392 [STAT4](#)¹⁰⁹. TGF β , also found at high levels in RA synovium induces persistent
393 expression of CXCR4 on synovial T cells, leading to their active CXCL12 mediated
394 retention, providing an additional mechanism for immune cell retention¹¹⁰. RA
395 synovial fibroblasts also release a repertoire of chemokines, generating a gradient
396 consisting of CCL2, CCL4, CCL5, CCL8, CXCL8, CXCL12 and IFN β ^{26,111,112}. This
397 chemokine gradient actively promotes the recruitment, retention and survival of

398 monocytes and CD4+ T cells at the inflamed synovial site (Figure 3). CXCL12,
399 VCAM-1 (CD106) and IL-6 therefore constitute part of a 'stromal address code',
400 critical for leukocyte survival and differentiation ²⁶.

401

402 *Endothelial cell cross talk*

403

404 Resident stromal cells populating inflamed synovium modulate the ability of
405 endothelial cells to recruit leukocytes via release of soluble mediators or direct cell-
406 cell contact. ~~Stromal~~ Fibroblasts isolated from healthy patients are known to
407 regulate the cytokine-sensitivity of vascular endothelium, while fibroblasts associated
408 with chronic inflammation adopt a pro-inflammatory phenotype ^{29,113}. Cytokine and
409 chemokine gradients mediate and sustain cross talk between endothelial cell,
410 synovial fibroblast and TRM populations. IL-6, TGF β 1 and VEGF released from TRM
411 provide the necessary cues to promote an angiogenic environment required to
412 sustain endothelial cell activation and dysfunction (Figure 3). This is supported by
413 antibody neutralisation of IL-6, which diminished the ability of endothelial cells to bind
414 lymphocytes in co-cultures with RA fibroblasts ²⁹.

415

416 The RA synovial fibroblast milieu further sustains an angiogenic environment through
417 chemokine gradients comprising CXCL1-5 and CXCL8 ²⁶ (Figure 3). RA fibroblasts
418 regulate expression of endothelial cell adhesion molecules, potentiate leukocyte
419 extravasation ⁵⁸ and induce unstimulated HUVEC to bind flowing lymphocytes via a
420 CXCR4-CXCL12 dependent manner ²⁹. Consequently, the interactions between cells
421 of mesenchymal origin create and sustain an inflammatory milieu, whereby synovial
422 inflammation persists and potentially becomes independent of its inciting cause. We
423 next consider how persistent inflammation culminates in tissue damage across soft
424 tissues that comprise the joint.

425

426 **2.3 Mesenchymal cells and their role in joint damage**

427

428 In health, early damage repair mechanisms maintain the integrity of joint soft tissues.
429 In joint disease, sustained inflammation, tissue remodeling and fibrosis ensue,
430 resulting in irreversible tissue damage. We next discuss how cells of mesenchymal

431 origin mediate fetal scarless healing and highlight the mechanisms by which they
432 induce damage across adult joint tissues.

433 In contrast to normal adult tissues, early human and murine fetal wounds and
434 wounds in Nude (FoxN1 deficient) mice heal without scar formation¹¹⁴. Fetal wounds
435 show diminished numbers of immune cells and lower levels of cytokines compared
436 to adult tissues¹¹⁵⁻¹¹⁸. Differences between embryonic and adult tissue healing are
437 also attributed to the milieu of pro-fibrotic growth factors released by TRM, including
438 those of the TGF β family. TGF β 1 levels are reduced and this growth factor shows
439 accelerated clearance in embryonic compared to adult tissue repair¹¹⁹⁻¹²¹.
440 Collectively these studies indicate a role for immune cell derived cytokines including
441 TNF α and TGF β in tissue scarring and healing¹²². Other studies highlight differences
442 between fetal and adult fibroblasts and localized production of MMP-9 and MMP-13
443 in the scarring process¹¹⁴. Fetal fibroblasts show enhanced synthetic function,
444 increased rate of turnover of collagen, hyaluronic acid, ECM components and
445 increased migration velocity compared to adult fibroblasts, suggesting rapid healing
446 may also play a role in scarless tissue repair¹²³⁻¹²⁵.

447 In adult tissues, fibroblasts and TRM directly contribute to joint destruction, bony
448 erosions and remodeling through expression of enzymes such as MMPs¹²⁶. MMP-2,
449 MMP-9 and MMP-13 have been specifically implicated in the pathogenesis of RA and
450 OA¹²⁷. MMP-9 is also upregulated by CXCL12 (SDF-1) a key chemokine secreted by
451 synovial fibroblasts¹²⁸. FAP is highly expressed within RA synovium and co-localises
452 with MMP-13, where it appears to play a role in tissue degradation¹²⁹. Cathepsins, a
453 major group of proteases involved in joint remodeling are also upregulated in the
454 diseased joint¹³⁰. Additionally fibroblasts can indirectly contribute through cross talk
455 with TRM and lymphocytes, further amplifying processes driving tissue damage
456 (Figure 3), whilst also presenting antigen to tissue infiltrating lymphocytes¹³¹.

457
458 Pathological conditions in which cells of mesenchymal origin play a role include
459 chronic inflammation (e.g. RA, chronic skin wound healing), tissue fibrosis (e.g.
460 COPD) and cancer (e.g. breast cancer). Interestingly, while these diseases differ
461 dramatically in aetiology and genetic predispositions, they converge in terms of
462 phenotype and function of the stromal component. Fibroblasts expand in the RA

463 synovial tissue and in the tumor parenchyma, while fibrosis is characterized by
464 profound changes in myofibroblast phenotype and function across different organs
465 such as the lungs and kidneys¹³². Whether these fibroblast properties are intrinsic
466 phenotypic changes acquired as a consequence of exposure to chronic
467 inflammation, or are derived from the conditioning of the pathogenic infiltrating cells
468 is still under investigation and seems to differ in the different conditions³⁷. Lafevre *et*
469 *al* reported epigenetically programmed aggressive cells may “spread” arthritis from
470 inflamed to uninfamed joints in the early stages of disease,¹³³. PDPN expressing
471 lining synovial fibroblasts are migratory and mediate release of cartilage destructive
472 MMPs^{33,62}. Collectively, these data raise the possibility of distinct mesenchymal cell
473 subsets implicated in mediating the effects of tissue damage in the diseased joint.
474 We next discuss how the possibility of selectively targeting pathogenic stromal
475 subpopulations mediating inflammation and tissue damage informs the development
476 of future strategies to successfully treat joint disease.

477

478 **3.0 Shaping the future landscape: therapeutic targeting of** 479 **mesenchymal cells**

480

481 Cells of mesenchymal origin including fibroblasts, TRM and endothelial cells
482 constitute the major cell types populating joint soft tissues. We have discussed the
483 roles and mechanisms by which these cells mediate joint inflammation, highlighting
484 their ability to act as immune sensing-sentinel cells, their capacity for activation,
485 positional memory and their altered phenotypes comprising multiple cellular sub-
486 populations. Multidirectional cross talk between stromal cell populations further fuels
487 the development of persistent inflammation. Given these important roles and
488 associated biological complexities, it is likely that residual disease activity in patients
489 treated with immune therapies may be attributable to stromal mediated inflammatory
490 responses, which are refractory to current therapies that target immune cell
491 populations¹³⁴. New therapeutic approaches are therefore required to ‘break the
492 cycle and reset the system’, particularly in scenarios where inflammation becomes
493 independent of the inciting stimulus. Given the limited capacity of joint tissues to
494 regenerate once damaged, there are significant challenges associated with curbing
495 tissue damage, which might be accomplished through moderating persistent

496 inflammation as a driver of fibrosis. We next discuss the requirement for future
497 strategies to address the pathobiology concerned with the stromal
498 microenvironment, targeting cells of mesenchymal origin. We review the drug
499 classes in current clinical use, those in early phase clinical trials and strategies with
500 pre-clinical potential to target stromal mediated joint disease. The cellular and
501 molecular targets and the mechanism of action through which these drug classes
502 function are summarized in Table 1.

503

504 *Existing licensed therapies*

505

506 Nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids provide
507 symptomatic relief for a broad array of conditions targeting inflammation and pain.
508 Their clinical use in the management of a multitude of diseases affecting the joint is
509 well established¹³⁵⁻¹³⁸. These therapies target fibroblasts, TRM and endothelial cells
510 via differing biological modes of action. Inhibition of COX activity by NSAIDs
511 dampens release of prostaglandins, leukotrienes and thromboxane A₂.
512 Corticosteroids act via the glucocorticoid receptor to inhibit cPLA₂, regulate
513 expression of NFκB / MAPK target genes and dampen release of inflammation
514 initiating eicosanoids. Whilst NSAIDs and corticosteroids continue to provide
515 background anti-inflammatory therapy for many rheumatic diseases, they are both
516 associated with well documented adverse systemic effects. Importantly, COX-2
517 selective NSAIDs also dampen protective endogenous resolution responses^{139,140},
518 which may paradoxically impede the capacity of inflamed joint tissues to heal.

519

520 Monoclonal antibodies enable precise molecular targeting of cytokines mediating
521 joint inflammation. The biological modes of action and efficacy of therapeutic
522 inhibitors of IL-1, IL-6, TNFα and IL-17 in current clinical use are well reported and
523 listed in Table 1. One disadvantage associated with selective cytokine inhibition is
524 the failure of this approach to fully target stromal mediated inflammatory responses
525 and address the complex multidirectional cross talk between mesenchymal cell
526 populations. Similarly targeting chemokine gradients is an attractive strategy to
527 moderate leukocyte retention¹⁴¹. However chemokine antagonists including
528 AMD3100 targeting CXCR4 are associated with adverse systemic effects¹⁴² and the

529 plethora of chemokines mediating stromal inflammatory responses presents a further
530 therapeutic challenge.

531

532 *Therapies in early phase clinical trials*

533

534 GM-CSF, predominantly produced by activated T cells, monocytes and
535 macrophages is also released by tissue resident cells of mesenchymal origin ¹⁴³.

536 Humanised IgG1 monoclonal antibodies to GM-CSF prevent interaction of this
537 cytokine with its receptor, reducing downstream signalling pathways converging on

538 NF κ B. GM-CSF has shown potential as a therapeutic target in autoimmune and
539 inflammatory disorders, including RA. ~~Early phase clinical trials demonstrated~~

540 ~~disease activity scores reduced in mavrilimumab treated patients with moderate RA.~~

541 Therapies targeting GM-CSF or its receptor have shown encouraging results in more
542 recent pre-clinical studies and are reviewed in detail elsewhere ¹⁴³. Recent phase IIb

543 studies have demonstrated that long term mavrilimumab treatment maintained

544 clinical responses and was well tolerated in RA patients with inadequate response to

545 DMARD's¹⁴⁴. Further investigation is required to determine the efficacy of GM-CSF

546 targeted therapies to modulate stromal mediated inflammatory responses in the joint.

547 Kinase inhibitors targeting JAK and SYK signalling pathways have been investigated
548 for their therapeutic utility to reduce cytokine release through JAK STAT ^{145,146} or

549 MAPK / PKC ^{147,148} blockade respectively (Table 1). Baricitinib, an oral reversible

550 inhibitor of JAK1 and JAK2 has shown therapeutic value in RA patients. This
551 treatment was associated with significant clinical improvements in patients with an

552 inadequate response to methotrexate compared with placebo and adalimumab
553 treated groups ¹⁴⁹. Protein kinase inhibitors target a broad range of cells types with

554 reported off target effects, highlighting the importance of understanding the
555 pharmacology of these drugs beyond the kinome ¹⁵⁰.

556

557 *Potential future strategies to target pathogenic stroma*

558

559 Developments in cancer medicine targeting cancer associated fibroblasts populating
560 tumour stroma have informed potential future strategies to target pathogenic stroma

561 in rheumatic disease ^{151,152}. Targeting pathogenic stroma presents a considerable

562 therapeutic challenge due to the biological complexity underpinning activation,
563 memory and phenotypic diversity exhibited by these mesenchymal cell populations.
564 Potential future strategies to treat residual rheumatic disease might include targeting
565 activated fibroblast subtypes, use of epigenetic modifiers or resolution agonists to
566 target stromal mediated inflammation. Pre-clinical evidence supporting these
567 approaches are discussed below.

568
569 Selective targeting of distinct fibroblast subtypes mediating joint inflammation and
570 tissue damage is a potential therapeutic strategy to target pathogenic stroma.
571 Cadherin-11 is known to regulate synovial fibroblast inflammation, synergizing with
572 IL-1 β and TNF α to regulate IL-6 release ¹⁵³. This study showed that cad-11 deficient
573 mice or anti-cad-11 mAb therapies reduced inflammation in arthritic mice,
574 suggesting that cadherin expression regulates the inflammatory capacity of synovial
575 fibroblasts. Cyclin dependent kinases regulate cell proliferation and survival via
576 specific inhibitors (CDKi) and are potential therapies to target fibroblast
577 **accumulationproliferation** in RA synovium (Table 1). CDK pathways become
578 dysregulated in cancer, leading to the development of anti-cancer drugs including
579 the CDKi Roscovitine ¹⁵⁴. In synovial fibroblasts, IL-6 and MMP-1 are known to be
580 regulated by CDKi p21 ¹⁵⁵. Given that CD34⁺CD90⁻ 'immunoregulatory' fibroblasts
581 are highly proliferative, invasive and produce IL-6⁶², CDKi therapies are a potential
582 strategy to target this fibroblast subset mediating joint disease.

583
584 We previously discussed how epigenetic changes are implicated in mediating
585 **stromal** fibroblast activation and memory. Epigenetic alterations in RA synovial
586 fibroblasts are listed in Table 1, identifying DNA methylation, histone modification
587 and miRNA as potential processes to therapeutically target ^{43,45-47,156}. Moderating the
588 epigenetic landscape is likely to have broad ranging effects on a variety of cell types,
589 with off target effects. Hence improved understanding of the pharmacology of these
590 drugs beyond the epigenome is essential before we can appreciate their potential
591 utility to treat joint disease.

592
593 The roles of proresolving mediators in joint health and disease are increasingly
594 understood, identifying resolution agonists as potential therapies to moderate joint

595 inflammation and promote tissue repair ⁹⁶. The biological modes of action of
596 proresolving mediators or ‘immunoresolvents’ are well established from *in vitro* and
597 *in vivo* studies and include limiting PMN infiltration, stimulating efferocytosis and
598 activation of endogenous tissue protective mechanisms ^{90-93,157,158}. Whilst
599 immunoresolvents target leukocytes, their biological actions are not associated with
600 immunosuppression ^{83,159}. Importantly, proresolving mediators also target fibroblasts,
601 TRM and endothelial cells types ¹⁶⁰⁻¹⁶² and therefore possess the capacity to
602 modulate stromal mediated inflammatory responses across joint tissues. Approaches
603 to potentiate resolution processes include dietary supplementation with proresolving
604 precursors, blocking catabolism of proresolving mediators or local delivery of stable
605 analogues binding proresolving receptors ⁹⁶. The pro-resolving mediator RvD3 was
606 found to limit leukocyte infiltration and paw joint eicosanoid levels in murine
607 inflammatory arthritis ¹⁶³. The stable epimer 17R-RvD1 significantly attenuated
608 arthritis severity, cachexia, paw oedema, leukocyte infiltration and shortened the
609 remission interval, showing cartilage protective actions in murine models of acute
610 inflammatory arthritis ¹⁶⁴. *In vitro* studies also highlight the capacity of 15-epi-LXA₄
611 and MaR1 stable epimers to regulate PDPN, STAT-1 and IL-6 in IL-1 β stimulated
612 diseased human tendon stromal cells ^{35,165}. Collectively these studies suggest
613 resolution pharmacology may be an important future therapeutic tool to address
614 stromal pathobiology in the joint.

615

616 **Conclusions**

617

618 Stromal cells of mesenchymal origin including fibroblasts, tissue resident
619 macrophages and endothelial cells are pivotal populations regulating health and
620 disease in musculoskeletal tissues. New insights are beginning to reveal the
621 mechanisms underpinning the activation and dysfunction of mesenchymal stromal
622 cells and their contribution to sustaining chronic joint inflammation. The discovery
623 that distinct synovial fibroblast subsets mediate joint inflammation and damage will
624 inform precision therapeutic targeting of pathogenic stromal cell populations. These
625 discoveries shape the future therapeutic landscape, presenting exciting new
626 approaches to address the pathogenic stromal microenvironment. Harnessing the
627 capacity to modulate cross talk between leukocyte and pathogenic stromal cell

628 populations is a critical barrier to overcome in our quest to advance therapeutic
629 strategies for patients with refractory joint disease.

630

631 **Glossary of terms**

632

633 **Mesoderm:** Middle embryonic primary germ layer residing between ectoderm and
634 endoderm

635

636 **Mesenchymal:** Embryonic connective tissue derived from the mesoderm

637

638 **Mesenchymal tissue:** Tissue of the musculoskeletal, circulatory and lymphatic
639 systems

640

641 **Stromal cell:** Non-haematopoietic, tissue resident cells.

642

643 **Stromal cell activation:** Process whereby stromal cells including fibroblasts, tissue
644 resident macrophages and endothelial cells adopt a pro-inflammatory phenotype and
645 express distinct molecular markers after exposure to an inflammatory stimulus.

646

647 **Stromal cell memory:** A change in the capacity of stromal cells to respond to
648 inflammatory stimuli

REFERENCES

- 1 Global Burden of Disease Study, C. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **386**, 743-800, doi:10.1016/S0140-6736(15)60692-4 (2015).
- 2 Crowley, T. *et al.* Priming in response to pro-inflammatory cytokines is a feature of adult synovial but not dermal fibroblasts. *Arthritis Res Ther* **19**, 35, doi:10.1186/s13075-017-1248-6 (2017).
- 3 Wolff, B., Burns, A. R., Middleton, J. & Rot, A. Endothelial cell "memory" of inflammatory stimulation: human venular endothelial cells store interleukin 8 in Weibel-Palade bodies. *J Exp Med* **188**, 1757-1762 (1998).
- 4 Murray, P. J. *et al.* Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. *Immunity* **41**, 14-20, doi:10.1016/j.immuni.2014.06.008 (2014).
- 5 Davies, L. C., Jenkins, S. J., Allen, J. E. & Taylor, P. R. Tissue-resident macrophages. *Nat Immunol* **14**, 986-995, doi:10.1038/ni.2705 (2013).
- 6 Davies, L. C. *et al.* Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation. *Nature communications* **4**, 1886, doi:10.1038/ncomms2877 (2013).
- 7 Epelman, S., Lavine, K. J. & Randolph, G. J. Origin and functions of tissue macrophages. *Immunity* **41**, 21-35, doi:10.1016/j.immuni.2014.06.013 (2014).
- 8 Hoeffel, G. & Ginhoux, F. Ontogeny of Tissue-Resident Macrophages. *Front Immunol* **6**, 486, doi:10.3389/fimmu.2015.00486 (2015).
- 9 Ginhoux, F. *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841-845, doi:10.1126/science.1194637 (2010).
- 10 Schulz, C. *et al.* A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* **336**, 86-90, doi:10.1126/science.1219179 (2012).
- 11 Yona, S. *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* **38**, 79-91, doi:10.1016/j.immuni.2012.12.001 (2013).
- 12 Hashimoto, D. *et al.* Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* **38**, 792-804, doi:10.1016/j.immuni.2013.04.004 (2013).
- 13 Guillemins, M. *et al.* Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med* **210**, 1977-1992, doi:10.1084/jem.20131199 (2013).
- 14 Jakubzick, C. *et al.* Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity* **39**, 599-610, doi:10.1016/j.immuni.2013.08.007 (2013).
- 15 Samokhvalov, I. M. Deconvoluting the ontogeny of hematopoietic stem cells. *Cell Mol Life Sci* **71**, 957-978, doi:10.1007/s00018-013-1364-7 (2014).
- 16 Jung, S. Macrophages and monocytes in 2017: Macrophages and monocytes: of tortoises and hares. *Nat Rev Immunol* **18**, 85-86, doi:10.1038/nri.2017.158 (2018).

- 17 Scherer, H. U., Huizinga, T. W. J., Kronke, G., Schett, G. & Toes, R. E. M. The B cell response to citrullinated antigens in the development of rheumatoid arthritis. *Nature reviews. Rheumatology* **14**, 157-169, doi:10.1038/nrrheum.2018.10 (2018).
- 18 Orr, C. *et al.* Synovial tissue research: a state-of-the-art review. *Nature reviews. Rheumatology* **13**, 463-475, doi:10.1038/nrrheum.2017.115 (2017).
- 19 Lubberts, E. The IL-23-IL-17 axis in inflammatory arthritis. *Nature reviews. Rheumatology* **11**, 562, doi:10.1038/nrrheum.2015.128 (2015).
- 20 Ospelt, C. Synovial fibroblasts in 2017. *RMD Open* **3**, e000471, doi:10.1136/rmdopen-2017-000471 (2017).
- 21 Filer, A., Raza, K., Salmon, M. & Buckley, C. D. Targeting stromal cells in chronic inflammation. *Discov Med* **7**, 20-26 (2007).
- 22 Tarin, D. & Croft, C. B. Ultrastructural features of wound healing in mouse skin. *J Anat* **105**, 189-190 (1969).
- 23 Abe, R., Donnelly, S. C., Peng, T., Bucala, R. & Metz, C. N. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol* **166**, 7556-7562 (2001).
- 24 Kalluri, R. & Neilson, E. G. Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* **112**, 1776-1784, doi:10.1172/JCI20530 (2003).
- 25 Iwano, M. *et al.* Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* **110**, 341-350, doi:10.1172/JCI15518 (2002).
- 26 Parsonage, G. *et al.* A stromal address code defined by fibroblasts. *Trends Immunol* **26**, 150-156, doi:10.1016/j.it.2004.11.014 (2005).
- 27 Marinkovich, M. P., Keene, D. R., Rimberg, C. S. & Burgeson, R. E. Cellular origin of the dermal-epidermal basement membrane. *Developmental dynamics : an official publication of the American Association of Anatomists* **197**, 255-267, doi:10.1002/aja.1001970404 (1993).
- 28 Sabatelli, P. *et al.* Collagen VI deficiency affects the organization of fibronectin in the extracellular matrix of cultured fibroblasts. *Matrix Biol* **20**, 475-486 (2001).
- 29 McGettrick, H. M. *et al.* Fibroblasts from different sites may promote or inhibit recruitment of flowing lymphocytes by endothelial cells. *Eur J Immunol* **39**, 113-125, doi:10.1002/eji.200838232 (2009).
- 30 Estell, E. G. *et al.* Fibroblast-like synoviocyte mechanosensitivity to fluid shear is modulated by interleukin-1alpha. *J Biomech* **60**, 91-99, doi:10.1016/j.jbiomech.2017.06.011 (2017).
- 31 Dakin, S. G. *et al.* Persistent stromal fibroblast activation is present in chronic tendinopathy. *Arthritis Res Ther* **Jan 25** (2017).
- 32 Choi, I. Y. *et al.* Stromal cell markers are differentially expressed in the synovial tissue of patients with early arthritis. *PLoS One* **12**, e0182751, doi:10.1371/journal.pone.0182751 (2017).
- 33 Croft, A. P. *et al.* Rheumatoid synovial fibroblasts differentiate into distinct subsets in the presence of cytokines and cartilage. *Arthritis Res Ther* **18**, 270, doi:10.1186/s13075-016-1156-1 (2016).
- 34 Wynn, T. A. & Vannella, K. M. Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity* **44**, 450-462, doi:10.1016/j.immuni.2016.02.015 (2016).

- 35 Dakin, S. G. *et al.* Inflammation activation and resolution in human tendon disease. *Science translational medicine* **7**, 311ra173, doi:10.1126/scitranslmed.aac4269 (2015).
- 36 Buckley, C. D. Why does chronic inflammation persist: An unexpected role for fibroblasts. *Immunol Lett* **138**, 12-14, doi:10.1016/j.imlet.2011.02.010 (2011).
- 37 Buckley, C. D. *et al.* Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. *Trends Immunol* **22**, 199-204 (2001).
- 38 Buckley, C. D., Barone, F., Nayar, S., Benezech, C. & Caamano, J. Stromal cells in chronic inflammation and tertiary lymphoid organ formation. *Annu Rev Immunol* **33**, 715-745, doi:10.1146/annurev-immunol-032713-120252 (2015).
- 39 Dang, Q., Liu, J., Li, J. & Sun, Y. Podoplanin: a novel regulator of tumor invasion and metastasis. *Med Oncol* **31**, 24, doi:10.1007/s12032-014-0024-6 (2014).
- 40 Zimmermann, T. *et al.* Isolation and characterization of rheumatoid arthritis synovial fibroblasts from primary culture--primary culture cells markedly differ from fourth-passage cells. *Arthritis Res* **3**, 72-76, doi:10.1186/ar142 (2001).
- 41 Juarez, M., Filer, A. & Buckley, C. D. Fibroblasts as therapeutic targets in rheumatoid arthritis and cancer. *Swiss Med Wkly* **142**, w13529, doi:10.4414/smw.2012.13529 (2012).
- 42 Patel, R., Filer, A., Barone, F. & Buckley, C. D. Stroma: fertile soil for inflammation. *Best Pract Res Clin Rheumatol* **28**, 565-576, doi:10.1016/j.berh.2014.10.022 (2014).
- 43 Ballestar, E. & Li, T. New insights into the epigenetics of inflammatory rheumatic diseases. *Nature reviews. Rheumatology* **13**, 593-605, doi:10.1038/nrrheum.2017.147 (2017).
- 44 Sohn, C. *et al.* Prolonged tumor necrosis factor alpha primes fibroblast-like synoviocytes in a gene-specific manner by altering chromatin. *Arthritis Rheumatol* **67**, 86-95, doi:10.1002/art.38871 (2015).
- 45 Karouzakis, E. *et al.* Analysis of early changes in DNA methylation in synovial fibroblasts of RA patients before diagnosis. *Sci Rep* **8**, 7370, doi:10.1038/s41598-018-24240-2 (2018).
- 46 Karouzakis, E., Gay, R. E., Michel, B. A., Gay, S. & Neidhart, M. DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum* **60**, 3613-3622, doi:10.1002/art.25018 (2009).
- 47 Gaur, N. *et al.* MicroRNAs interfere with DNA methylation in rheumatoid arthritis synovial fibroblasts. *RMD Open* **2**, e000299, doi:10.1136/rmdopen-2016-000299 (2016).
- 48 Seeley, J. J. & Ghosh, S. Molecular mechanisms of innate memory and tolerance to LPS. *J Leukoc Biol* **101**, 107-119, doi:10.1189/jlb.3MR0316-118RR (2017).
- 49 Kawasaki, T. & Kawai, T. Toll-like receptor signaling pathways. *Front Immunol* **5**, 461, doi:10.3389/fimmu.2014.00461 (2014).
- 50 Crowley, T., Buckley, C. D. & Clark, A. R. Stroma: the forgotten cells of innate immune memory. *Clin Exp Immunol*, doi:10.1111/cei.13149 (2018).
- 51 Heinemeier, K. M., Schjerling, P., Heinemeier, J., Magnusson, S. P. & Kjaer, M. Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb 14C. *FASEB J* **27**, 2074-2079, doi:10.1096/fj.12-225599 (2013).
- 52 Klein, K. *et al.* The epigenetic architecture at gene promoters determines cell type-specific LPS tolerance. *J Autoimmun* **83**, 122-133, doi:10.1016/j.jaut.2017.07.001 (2017).

- 53 Koch, S. R., Lamb, F. S., Hellman, J., Sherwood, E. R. & Stark, R. J. Potentiation and tolerance of toll-like receptor priming in human endothelial cells. *Transl Res* **180**, 53-67 e54, doi:10.1016/j.trsl.2016.08.001 (2017).
- 54 Ospelt, C. & Frank-Bertoncelj, M. Why location matters - site-specific factors in rheumatic diseases. *Nature reviews. Rheumatology* **13**, 433-442, doi:10.1038/nrrheum.2017.96 (2017).
- 55 Ospelt, C., Reedquist, K. A., Gay, S. & Tak, P. P. Inflammatory memories: is epigenetics the missing link to persistent stromal cell activation in rheumatoid arthritis? *Autoimmun Rev* **10**, 519-524, doi:10.1016/j.autrev.2011.04.001 (2011).
- 56 Frank-Bertoncelj, M. *et al.* Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nature communications* **8**, 14852, doi:10.1038/ncomms14852 (2017).
- 57 Sherlock, J. P. *et al.* IL-23 induces spondyloarthritis by acting on ROR-gammat+ CD3+CD4-CD8- enthesal resident T cells. *Nat Med* **18**, 1069-1076, doi:10.1038/nm.2817 (2012).
- 58 Filer, A. *et al.* Identification of a transitional fibroblast function in very early rheumatoid arthritis. *Ann Rheum Dis* **76**, 2105-2112, doi:10.1136/annrheumdis-2017-211286 (2017).
- 59 Alexander, T., Nolte, C. & Krumlauf, R. Hox genes and segmentation of the hindbrain and axial skeleton. *Annu Rev Cell Dev Biol* **25**, 431-456, doi:10.1146/annurev.cellbio.042308.113423 (2009).
- 60 Krumlauf, R. Hox genes in vertebrate development. *Cell* **78**, 191-201 (1994).
- 61 Ospelt, C., Gay, S. & Klein, K. Epigenetics in the pathogenesis of RA. *Semin Immunopathol* **39**, 409-419, doi:10.1007/s00281-017-0621-5 (2017).
- 62 Mizoguchi, F. *et al.* Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nature communications* **9**, 789, doi:10.1038/s41467-018-02892-y (2018).
- 63 Stephenson, W. *et al.* Single-cell RNA-seq of rheumatoid arthritis synovial tissue using low-cost microfluidic instrumentation. *Nature communications* **9**, 791, doi:10.1038/s41467-017-02659-x (2018).
- 64 Jackson, J. R., Seed, M. P., Kircher, C. H., Willoughby, D. A. & Winkler, J. D. The codependence of angiogenesis and chronic inflammation. *FASEB J* **11**, 457-465 (1997).
- 65 Ley, K. & Reuterman, J. Leucocyte-endothelial interactions in health and disease. *Handb Exp Pharmacol*, 97-133 (2006).
- 66 Bazzoni, G. Endothelial tight junctions: permeable barriers of the vessel wall. *Thromb Haemost* **95**, 36-42 (2006).
- 67 Pober, J. S. & Sessa, W. C. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol* **7**, 803-815, doi:10.1038/nri2171 (2007).
- 68 Weber, A. J., De Bandt, M. & Gaudry, M. Immunohistochemical analysis of vascular endothelial growth factor expression in severe and destructive rheumatoid arthritis. *J Rheumatol* **27**, 2284-2286 (2000).
- 69 Girard, J. P. & Springer, T. A. High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. *Immunol Today* **16**, 449-457 (1995).
- 70 Middleton, J. *et al.* Endothelial cell phenotypes in the rheumatoid synovium: activated, angiogenic, apoptotic and leaky. *Arthritis Res Ther* **6**, 60-72, doi:10.1186/ar1156 (2004).
- 71 Johnson, B. A., Haines, G. K., Harlow, L. A. & Koch, A. E. Adhesion molecule expression in human synovial tissue. *Arthritis Rheum* **36**, 137-146 (1993).

- 72 Szekanecz, Z. *et al.* Differential distribution of intercellular adhesion molecules (ICAM-1, ICAM-2, and ICAM-3) and the MS-1 antigen in normal and diseased human synovia. Their possible pathogenetic and clinical significance in rheumatoid arthritis. *Arthritis Rheum* **37**, 221-231 (1994).
- 73 Wilkinson, L. S., Edwards, J. C., Poston, R. N. & Haskard, D. O. Expression of vascular cell adhesion molecule-1 in normal and inflamed synovium. *Lab Invest* **68**, 82-88 (1993).
- 74 Bordy, R. *et al.* Microvascular endothelial dysfunction in rheumatoid arthritis. *Nature reviews. Rheumatology* **14**, 404-420, doi:10.1038/s41584-018-0022-8 (2018).
- 75 Liao, J. K. Linking endothelial dysfunction with endothelial cell activation. *J Clin Invest* **123**, 540-541, doi:10.1172/JCI66843 (2013).
- 76 Chyou, S. *et al.* Fibroblast-type reticular stromal cells regulate the lymph node vasculature. *J Immunol* **181**, 3887-3896 (2008).
- 77 Alitalo, K. The lymphatic vasculature in disease. *Nat Med* **17**, 1371-1380, doi:10.1038/nm.2545 (2011).
- 78 Louveau, A. *et al.* Structural and functional features of central nervous system lymphatic vessels. *Nature* **523**, 337-341, doi:10.1038/nature14432 (2015).
- 79 Bouta, E. M. *et al.* Targeting lymphatic function as a novel therapeutic intervention for rheumatoid arthritis. *Nature reviews. Rheumatology* **14**, 94-106, doi:10.1038/nrrheum.2017.205 (2018).
- 80 Zheng, W., Aspelund, A. & Alitalo, K. Lymphangiogenic factors, mechanisms, and applications. *J Clin Invest* **124**, 878-887, doi:10.1172/JCI71603 (2014).
- 81 Zhou, Q., Wood, R., Schwarz, E. M., Wang, Y. J. & Xing, L. Near-infrared lymphatic imaging demonstrates the dynamics of lymph flow and lymphangiogenesis during the acute versus chronic phases of arthritis in mice. *Arthritis Rheum* **62**, 1881-1889, doi:10.1002/art.27464 (2010).
- 82 Rahimi, H. *et al.* Lymphatic imaging to assess rheumatoid flare: mechanistic insights and biomarker potential. *Arthritis Res Ther* **18**, 194, doi:10.1186/s13075-016-1092-0 (2016).
- 83 Fullerton, J. N. & Gilroy, D. W. Resolution of inflammation: a new therapeutic frontier. *Nat Rev Drug Discov* **15**, 551-567, doi:10.1038/nrd.2016.39 (2016).
- 84 Kennedy, A., Fearon, U., Veale, D. J. & Godson, C. Macrophages in synovial inflammation. *Front Immunol* **2**, 52, doi:10.3389/fimmu.2011.00052 (2011).
- 85 Burmester, G. R., Stuhlmuller, B., Keyszer, G. & Kinne, R. W. Mononuclear phagocytes and rheumatoid synovitis. Mastermind or workhorse in arthritis? *Arthritis Rheum* **40**, 5-18 (1997).
- 86 Vallejo, A. N., Yang, H., Klimiuk, P. A., Weyand, C. M. & Goronzy, J. J. Synoviocyte-mediated expansion of inflammatory T cells in rheumatoid synovitis is dependent on CD47-thrombospondin 1 interaction. *J Immunol* **171**, 1732-1740 (2003).
- 87 Abeles, A. M. & Pillinger, M. H. The role of the synovial fibroblast in rheumatoid arthritis: cartilage destruction and the regulation of matrix metalloproteinases. *Bull NYU Hosp Jt Dis* **64**, 20-24 (2006).
- 88 Misharin, A. V. *et al.* Nonclassical Ly6C(-) monocytes drive the development of inflammatory arthritis in mice. *Cell Rep* **9**, 591-604, doi:10.1016/j.celrep.2014.09.032 (2014).
- 89 Mandelin, A. M., 2nd *et al.* Transcriptional Profiling of Synovial Macrophages Using Minimally Invasive Ultrasound-Guided Synovial Biopsies in Rheumatoid Arthritis. *Arthritis Rheumatol* **70**, 841-854, doi:10.1002/art.40453 (2018).

- 90 Serhan, C. N., Hamberg, M. & Samuelsson, B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proc Natl Acad Sci U S A* **81**, 5335-5339 (1984).
- 91 Serhan, C. N., Levy, B. D., Clish, C. B., Gronert, K. & Chiang, N. Lipoxins, aspirin-triggered 15-epi-lipoxin stable analogs and their receptors in anti-inflammation: a window for therapeutic opportunity. *Ernst Schering Res Found Workshop*, 143-185 (2000).
- 92 Serhan, C. N., Gotlinger, K., Hong, S. & Arita, M. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. *Prostaglandins Other Lipid Mediat* **73**, 155-172 (2004).
- 93 Serhan, C. N. *et al.* Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med* **206**, 15-23, doi:jem.20081880 [pii] 10.1084/jem.20081880 (2009).
- 94 Serhan, C. N. *et al.* Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. *J Immunol* **176**, 1848-1859 (2006).
- 95 Serhan, C. N., Chiang, N. & Van Dyke, T. E. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* **8**, 349-361, doi:nri2294 [pii] 10.1038/nri2294 (2008).
- 96 Perretti, M., Cooper, D., Dalli, J. & Norling, L. V. Immune resolution mechanisms in inflammatory arthritis. *Nature reviews. Rheumatology* **13**, 87-99, doi:10.1038/nrrheum.2016.193 (2017).
- 97 Stables, M. J. *et al.* Transcriptomic analyses of murine resolution-phase macrophages. *Blood* **118**, e192-208, doi:10.1182/blood-2011-04-345330 (2011).
- 98 Millar, N. L., Murrell, G. A. & McInnes, I. B. Inflammatory mechanisms in tendinopathy - towards translation. *Nature reviews. Rheumatology* **13**, 110-122, doi:10.1038/nrrheum.2016.213 (2017).
- 99 Nefla, M., Holzinger, D., Berenbaum, F. & Jacques, C. The danger from within: alarmins in arthritis. *Nature reviews. Rheumatology* **12**, 669-683, doi:10.1038/nrrheum.2016.162 (2016).
- 100 Pisetsky, D. S., Erlandsson-Harris, H. & Andersson, U. High-mobility group box protein 1 (HMGB1): an alarmin mediating the pathogenesis of rheumatic disease. *Arthritis Res Ther* **10**, 209, doi:10.1186/ar2440 (2008).
- 101 Carrion, M. *et al.* IL-22/IL-22R1 axis and S100A8/A9 alarmins in human osteoarthritic and rheumatoid arthritis synovial fibroblasts. *Rheumatology (Oxford)* **52**, 2177-2186, doi:10.1093/rheumatology/ket315 (2013).
- 102 Zuliani-Alvarez, L. *et al.* Mapping tenascin-C interaction with toll-like receptor 4 reveals a new subset of endogenous inflammatory triggers. *Nature communications* **8**, 1595, doi:10.1038/s41467-017-01718-7 (2017).
- 103 Lee, G. *et al.* Fully reduced HMGB1 accelerates the regeneration of multiple tissues by transitioning stem cells to GAlert. *Proc Natl Acad Sci U S A* **115**, E4463-E4472, doi:10.1073/pnas.1802893115 (2018).
- 104 Burman, A. *et al.* A chemokine-dependent stromal induction mechanism for aberrant lymphocyte accumulation and compromised lymphatic return in rheumatoid arthritis. *J Immunol* **174**, 1693-1700 (2005).

- 105 Buckley, C. D. & McGettrick, H. M. Leukocyte trafficking between stromal compartments: lessons from rheumatoid arthritis. *Nature reviews. Rheumatology*, doi:10.1038/s41584-018-0042-4 (2018).
- 106 Kalinski, P. Regulation of immune responses by prostaglandin E2. *J Immunol* **188**, 21-28, doi:10.4049/jimmunol.1101029 (2012).
- 107 Ivanov, I. et al. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* **126**, 1121-1133, doi:10.1016/j.cell.2006.07.035 (2006).
- 108 Fleetwood, A. J., Lawrence, T., Hamilton, J. A. & Cook, A. D. Granulocyte-macrophage colony-stimulating factor (CSF) and macrophage CSF-dependent macrophage phenotypes display differences in cytokine profiles and transcription factor activities: implications for CSF blockade in inflammation. *J Immunol* **178**, 5245-5252 (2007).
- 109 Nguyen, H. N. et al. Autocrine Loop Involving IL-6 Family Member LIF, LIF Receptor, and STAT4 Drives Sustained Fibroblast Production of Inflammatory Mediators. *Immunity* **46**, 220-232, doi:10.1016/j.immuni.2017.01.004 (2017).
- 110 Buckley, C. D. et al. Persistent induction of the chemokine receptor CXCR4 by TGF- β 1 on synovial T cells contributes to their accumulation within the rheumatoid synovium. *J Immunol* **165**, 3423-3429 (2000).
- 111 Bradfield, P. F. et al. Rheumatoid fibroblast-like synoviocytes overexpress the chemokine stromal cell-derived factor 1 (CXCL12), which supports distinct patterns and rates of CD4+ and CD8+ T cell migration within synovial tissue. *Arthritis Rheum* **48**, 2472-2482, doi:10.1002/art.11219 (2003).
- 112 Filer, A. et al. Differential survival of leukocyte subsets mediated by synovial, bone marrow, and skin fibroblasts: site-specific versus activation-dependent survival of T cells and neutrophils. *Arthritis Rheum* **54**, 2096-2108, doi:10.1002/art.21930 (2006).
- 113 Nash, G. B., Buckley, C. D. & Ed Rainger, G. The local physicochemical environment conditions the proinflammatory response of endothelial cells and thus modulates leukocyte recruitment. *FEBS Lett* **569**, 13-17, doi:10.1016/j.febslet.2004.05.040 (2004).
- 114 Gawronska-Kozak, B., Bogacki, M., Rim, J. S., Monroe, W. T. & Manuel, J. A. Scarless skin repair in immunodeficient mice. *Wound Repair Regen* **14**, 265-276, doi:10.1111/j.1743-6109.2006.00121.x (2006).
- 115 Cowin, A. J., Brosnan, M. P., Holmes, T. M. & Ferguson, M. W. Endogenous inflammatory response to dermal wound healing in the fetal and adult mouse. *Developmental dynamics : an official publication of the American Association of Anatomists* **212**, 385-393, doi:10.1002/(SICI)1097-0177(199807)212:3<385::AID-AJA6>3.0.CO;2-D (1998).
- 116 Hopkinson-Woolley, J., Hughes, D., Gordon, S. & Martin, P. Macrophage recruitment during limb development and wound healing in the embryonic and foetal mouse. *J Cell Sci* **107 (Pt 5)**, 1159-1167 (1994).
- 117 Cowin, A. J., Holmes, T. M., Brosnan, P. & Ferguson, M. W. Expression of TGF- β and its receptors in murine fetal and adult dermal wounds. *Eur J Dermatol* **11**, 424-431 (2001).
- 118 Rolfe, K. J. & Grobbelaar, A. O. A review of fetal scarless healing. *ISRN Dermatol* **2012**, 698034, doi:10.5402/2012/698034 (2012).
- 119 Whitby, D. J. & Ferguson, M. W. Immunohistochemical localization of growth factors in fetal wound healing. *Dev Biol* **147**, 207-215 (1991).

- 120 Martin, P., Dickson, M. C., Millan, F. A. & Akhurst, R. J. Rapid induction and clearance of TGF beta 1 is an early response to wounding in the mouse embryo. *Dev Genet* **14**, 225-238, doi:10.1002/dvg.1020140309 (1993).
- 121 Frank, S., Madlener, M. & Werner, S. Transforming growth factors beta1, beta2, and beta3 and their receptors are differentially regulated during normal and impaired wound healing. *J Biol Chem* **271**, 10188-10193 (1996).
- 122 Li, Z. *et al.* Epidermal Notch1 recruits RORgamma(+) group 3 innate lymphoid cells to orchestrate normal skin repair. *Nature communications* **7**, 11394, doi:10.1038/ncomms11394 (2016).
- 123 Coolen, N. A., Schouten, K. C., Boekema, B. K., Middelkoop, E. & Ulrich, M. M. Wound healing in a fetal, adult, and scar tissue model: a comparative study. *Wound Repair Regen* **18**, 291-301, doi:10.1111/j.1524-475X.2010.00585.x (2010).
- 124 Coolen, N. A., Schouten, K. C., Middelkoop, E. & Ulrich, M. M. Comparison between human fetal and adult skin. *Arch Dermatol Res* **302**, 47-55, doi:10.1007/s00403-009-0989-8 (2010).
- 125 Stalling, S. S. & Nicoll, S. B. Fetal ACL fibroblasts exhibit enhanced cellular properties compared with adults. *Clin Orthop Relat Res* **466**, 3130-3137, doi:10.1007/s11999-008-0391-4 (2008).
- 126 Clark, I. M., Powell, L. K., Ramsey, S., Hazleman, B. L. & Cawston, T. E. The measurement of collagenase, tissue inhibitor of metalloproteinases (TIMP), and collagenase-TIMP complex in synovial fluids from patients with osteoarthritis and rheumatoid arthritis. *Arthritis Rheum* **36**, 372-379 (1993).
- 127 Sato, H. *et al.* A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* **370**, 61-65, doi:10.1038/370061a0 (1994).
- 128 Grassi, F. *et al.* CXCL12 chemokine up-regulates bone resorption and MMP-9 release by human osteoclasts: CXCL12 levels are increased in synovial and bone tissue of rheumatoid arthritis patients. *J Cell Physiol* **199**, 244-251, doi:10.1002/jcp.10445 (2004).
- 129 Bauer, S. *et al.* Fibroblast activation protein is expressed by rheumatoid myofibroblast-like synoviocytes. *Arthritis Res Ther* **8**, R171, doi:10.1186/ar2080 (2006).
- 130 Huet, G. *et al.* Measurement of elastase and cysteine proteinases in synovial fluid of patients with rheumatoid arthritis, sero-negative spondylarthropathies, and osteoarthritis. *Clin Chem* **38**, 1694-1697 (1992).
- 131 Carmona-Rivera, C. *et al.* Synovial fibroblast-neutrophil interactions promote pathogenic adaptive immunity in rheumatoid arthritis. *Sci Immunol* **2**, doi:10.1126/sciimmunol.aag3358 (2017).
- 132 Brabletz, T., Kalluri, R., Nieto, M. A. & Weinberg, R. A. EMT in cancer. *Nat Rev Cancer* **18**, 128-134, doi:10.1038/nrc.2017.118 (2018).
- 133 Lefevre, S. *et al.* Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nat Med* **15**, 1414-1420, doi:10.1038/nm.2050 (2009).
- 134 Jutley, G., Raza, K. & Buckley, C. D. New pathogenic insights into rheumatoid arthritis. *Curr Opin Rheumatol* **27**, 249-255, doi:10.1097/BOR.000000000000174 (2015).
- 135 Whittle, S. L. *et al.* Multinational evidence-based recommendations for pain management by pharmacotherapy in inflammatory arthritis: integrating systematic literature research and expert opinion of a broad panel of rheumatologists in the 3e Initiative. *Rheumatology (Oxford)* **51**, 1416-1425, doi:10.1093/rheumatology/kes032 (2012).

- 136 FitzGerald, G. A. & Patrono, C. The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med* **345**, 433-442, doi:10.1056/NEJM200108093450607 (2001).
- 137 Gotzsche, P. C. & Johansen, H. K. Meta-analysis of short-term low dose prednisolone versus placebo and non-steroidal anti-inflammatory drugs in rheumatoid arthritis. *BMJ* **316**, 811-818 (1998).
- 138 Wassenberg, S., Rau, R., Steinfeld, P. & Zeidler, H. Very low-dose prednisolone in early rheumatoid arthritis retards radiographic progression over two years: a multicenter, double-blind, placebo-controlled trial. *Arthritis Rheum* **52**, 3371-3380, doi:10.1002/art.21421 (2005).
- 139 Gilroy, D. W. *et al.* Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* **5**, 698-701, doi:10.1038/9550 (1999).
- 140 Gilroy, D. W., Lawrence, T., Perretti, M. & Rossi, A. G. Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov* **3**, 401-416, doi:10.1038/nrd1383 nrd1383 [pii] (2004).
- 141 Scholten, D. J. *et al.* Pharmacological modulation of chemokine receptor function. *Br J Pharmacol* **165**, 1617-1643, doi:10.1111/j.1476-5381.2011.01551.x (2012).
- 142 Filer, A. The fibroblast as a therapeutic target in rheumatoid arthritis. *Current opinion in pharmacology* **13**, 413-419, doi:10.1016/j.coph.2013.02.006 (2013).
- 143 Wicks, I. P. & Roberts, A. W. Targeting GM-CSF in inflammatory diseases. *Nature reviews. Rheumatology* **12**, 37-48, doi:10.1038/nrrheum.2015.161 (2016).
- 144 Burmester, G. R. *et al.* Mavrilimumab, a Fully Human Granulocyte-Macrophage Colony-Stimulating Factor Receptor alpha Monoclonal Antibody: Long-Term Safety and Efficacy in Patients With Rheumatoid Arthritis. *Arthritis Rheumatol* **70**, 679-689, doi:10.1002/art.40420 (2018).
- 145 Kremer, J. M. *et al.* The safety and efficacy of a JAK inhibitor in patients with active rheumatoid arthritis: Results of a double-blind, placebo-controlled phase IIa trial of three dosage levels of CP-690,550 versus placebo. *Arthritis Rheum* **60**, 1895-1905, doi:10.1002/art.24567 (2009).
- 146 Fleischmann, R. *et al.* Placebo-controlled trial of tofacitinib monotherapy in rheumatoid arthritis. *N Engl J Med* **367**, 495-507, doi:10.1056/NEJMoa1109071 (2012).
- 147 Weinblatt, M. E. *et al.* Treatment of rheumatoid arthritis with a Syk kinase inhibitor: a twelve-week, randomized, placebo-controlled trial. *Arthritis Rheum* **58**, 3309-3318, doi:10.1002/art.23992 (2008).
- 148 Weinblatt, M. E. *et al.* An oral spleen tyrosine kinase (Syk) inhibitor for rheumatoid arthritis. *N Engl J Med* **363**, 1303-1312, doi:10.1056/NEJMoa1000500 (2010).
- 149 Taylor, P. C. *et al.* Baricitinib versus Placebo or Adalimumab in Rheumatoid Arthritis. *N Engl J Med* **376**, 652-662, doi:10.1056/NEJMoa1608345 (2017).
- 150 Munoz, L. Non-kinase targets of protein kinase inhibitors. *Nat Rev Drug Discov* **16**, 424-440, doi:10.1038/nrd.2016.266 (2017).
- 151 Sund, M. & Kalluri, R. Tumor stroma derived biomarkers in cancer. *Cancer Metastasis Rev* **28**, 177-183, doi:10.1007/s10555-008-9175-2 (2009).
- 152 Sherlock, J. P., Filer, A. D., Isaacs, J. D. & Buckley, C. D. What can rheumatologists learn from translational cancer therapy? *Arthritis Res Ther* **15**, 114, doi:10.1186/ar4203 (2013).

- 153 Chang, S. K. *et al.* Cadherin-11 regulates fibroblast inflammation. *Proc Natl Acad Sci U S A* **108**, 8402-8407, doi:10.1073/pnas.1019437108 (2011).
- 154 Nair, B. C., Vallabhaneni, S., Tekmal, R. R. & Vadlamudi, R. K. Roscovitine confers tumor suppressive effect on therapy-resistant breast tumor cells. *Breast Cancer Res* **13**, R80, doi:10.1186/bcr2929 (2011).
- 155 Perlman, H. *et al.* IL-6 and matrix metalloproteinase-1 are regulated by the cyclin-dependent kinase inhibitor p21 in synovial fibroblasts. *J Immunol* **170**, 838-845 (2003).
- 156 Hammaker, D. *et al.* LBH Gene Transcription Regulation by the Interplay of an Enhancer Risk Allele and DNA Methylation in Rheumatoid Arthritis. *Arthritis Rheumatol* **68**, 2637-2645, doi:10.1002/art.39746 (2016).
- 157 Norris, P. C., Libreros, S., Chiang, N. & Serhan, C. N. A cluster of immunoresolvents links coagulation to innate host defense in human blood. *Sci Signal* **10**, doi:10.1126/scisignal.aan1471 (2017).
- 158 Serhan, C. N. Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. *Mol Aspects Med* **58**, 1-11, doi:10.1016/j.mam.2017.03.001 (2017).
- 159 Serhan, C. N. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* **510**, 92-101, doi:10.1038/nature13479 (2014).
- 160 Herrera, B. S. *et al.* LXA4 actions direct fibroblast function and wound closure. *Biochem Biophys Res Commun* **464**, 1072-1077, doi:10.1016/j.bbrc.2015.07.076 (2015).
- 161 Tabas, I. & Glass, C. K. Anti-inflammatory therapy in chronic disease: challenges and opportunities. *Science* **339**, 166-172, doi:10.1126/science.1230720 (2013).
- 162 Merched, A. J., Ko, K., Gotlinger, K. H., Serhan, C. N. & Chan, L. Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. *FASEB J* **22**, 3595-3606, doi:10.1096/fj.08-112201 (2008).
- 163 Arnardottir, H. H. *et al.* Resolvin D3 Is Dysregulated in Arthritis and Reduces Arthritic Inflammation. *J Immunol* **197**, 2362-2368, doi:10.4049/jimmunol.1502268 (2016).
- 164 Norling, L. V. *et al.* Proresolving and cartilage-protective actions of resolvin D1 in inflammatory arthritis. *JCI Insight* **1**, e85922, doi:10.1172/jci.insight.85922 (2016).
- 165 Dakin, S. G. *et al.* Increased 15-PGDH expression leads to dysregulated resolution responses in stromal cells from patients with chronic tendinopathy. *Sci Rep* **7**, 11009, doi:10.1038/s41598-017-11188-y (2017).
- 166 Bresnihan, B. *et al.* Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *Arthritis Rheum* **41**, 2196-2204, doi:10.1002/1529-0131(199812)41:12<2196::AID-ART15>3.0.CO;2-2 (1998).
- 167 Jiang, Y. *et al.* A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: radiologic progression and correlation of Genant and Larsen scores. *Arthritis Rheum* **43**, 1001-1009, doi:10.1002/1529-0131(200005)43:5<1001::AID-ANR7>3.0.CO;2-P (2000).
- 168 Nishimoto, N. *et al.* Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): evidence of clinical and radiographic benefit from an x ray reader-blinded randomised controlled trial

- of tocilizumab. *Ann Rheum Dis* **66**, 1162-1167, doi:10.1136/ard.2006.068064 (2007).
- 169 Nishimoto, N. *et al.* Study of active controlled tocilizumab monotherapy for rheumatoid arthritis patients with an inadequate response to methotrexate (SATORI): significant reduction in disease activity and serum vascular endothelial growth factor by IL-6 receptor inhibition therapy. *Mod Rheumatol* **19**, 12-19, doi:10.1007/s10165-008-0125-1 (2009).
- 170 Genovese, M. C. *et al.* Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: the tocilizumab in combination with traditional disease-modifying antirheumatic drug therapy study. *Arthritis Rheum* **58**, 2968-2980, doi:10.1002/art.23940 (2008).
- 171 Emery, P. *et al.* IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. *Ann Rheum Dis* **67**, 1516-1523, doi:10.1136/ard.2008.092932 (2008).
- 172 Smolen, J. S. *et al.* Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet* **371**, 987-997, doi:10.1016/S0140-6736(08)60453-5 (2008).
- 173 Bathon, J. M. *et al.* A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. *N Engl J Med* **343**, 1586-1593, doi:10.1056/NEJM200011303432201 (2000).
- 174 Moreland, L. W. *et al.* Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N Engl J Med* **337**, 141-147, doi:10.1056/NEJM199707173370301 (1997).
- 175 Weinblatt, M. E. *et al.* A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* **340**, 253-259, doi:10.1056/NEJM199901283400401 (1999).
- 176 Lipsky, P. E. *et al.* Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med* **343**, 1594-1602, doi:10.1056/NEJM200011303432202 (2000).
- 177 Keystone, E. C. *et al.* Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. *Arthritis Rheum* **50**, 1400-1411, doi:10.1002/art.20217 (2004).
- 178 van de Putte, L. B. *et al.* Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. *Ann Rheum Dis* **63**, 508-516, doi:10.1136/ard.2003.013052 (2004).
- 179 Burmester, G. R. *et al.* Mavrilimumab, a human monoclonal antibody targeting GM-CSF receptor-alpha, in subjects with rheumatoid arthritis: a randomised, double-blind, placebo-controlled, phase I, first-in-human study. *Ann Rheum Dis* **70**, 1542-1549, doi:10.1136/ard.2010.146225 (2011).
- 180 Avci, A. B., Feist, E. & Burmester, G. R. Targeting GM-CSF in rheumatoid arthritis. *Clin Exp Rheumatol* **34**, 39-44 (2016).

- 181 Langley, R. G. *et al.* Secukinumab in plaque psoriasis--results of two phase 3 trials. *N Engl J Med* **371**, 326-338, doi:10.1056/NEJMoa1314258 (2014).
- 182 Mrowietz, U. *et al.* Secukinumab retreatment-as-needed versus fixed-interval maintenance regimen for moderate to severe plaque psoriasis: A randomized, double-blind, noninferiority trial (SCULPTURE). *J Am Acad Dermatol* **73**, 27-36 e21, doi:10.1016/j.jaad.2015.04.011 (2015).
- 183 Blauvelt, A. *et al.* Secukinumab is superior to ustekinumab in clearing skin of subjects with moderate-to-severe plaque psoriasis up to 1 year: Results from the CLEAR study. *J Am Acad Dermatol* **76**, 60-69 e69, doi:10.1016/j.jaad.2016.08.008 (2017).
- 184 Lee, A. *et al.* Tumor necrosis factor alpha induces sustained signaling and a prolonged and unremitting inflammatory response in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum* **65**, 928-938, doi:10.1002/art.37853 (2013).
- 185 Lin, J. *et al.* A novel p53/microRNA-22/Cyr61 axis in synovial cells regulates inflammation in rheumatoid arthritis. *Arthritis Rheumatol* **66**, 49-59, doi:10.1002/art.38142 (2014).
- 186 Philippe, L. *et al.* MiR-20a regulates ASK1 expression and TLR4-dependent cytokine release in rheumatoid fibroblast-like synoviocytes. *Ann Rheum Dis* **72**, 1071-1079, doi:10.1136/annrheumdis-2012-201654 (2013).
- 187 Stanczyk, J. *et al.* Altered expression of microRNA-203 in rheumatoid arthritis synovial fibroblasts and its role in fibroblast activation. *Arthritis Rheum* **63**, 373-381, doi:10.1002/art.30115 (2011).
- 188 Headland, S. E. *et al.* Neutrophil-derived microvesicles enter cartilage and protect the joint in inflammatory arthritis. *Science translational medicine* **7**, 315ra190, doi:10.1126/scitranslmed.aac5608 (2015).

Table 1: Drugs to target the pathogenic stroma and associated immune cell cross talk in joint disease

Drug Class	Target Mesenchymal Cell	Molecular Target	Mechanism of Action	References
NSAIDs	Fibroblast (F) Tissue Resident Macrophage (TRM) Endothelial Cell (EC)	COX-1 COX-2	Selective / non-selective inhibition of COX to reduce release of prostaglandins, leukotrienes, thromboxane	135,136
Corticosteroids	F, TRM, EC	glucocorticoid receptor	cPLA2 inhibition regulate NFκB / MAPK target genes reduce prostaglandins, leukotrienes, thromboxane	137,138
Monoclonal Ab				
IL-1	TRM (F)	IL-1R	Reduce effects of inflammasome and caspase activation	166,167
IL-6	TRM, F	IL-6R	Reduce STAT-3 signalling	168-172
TNF	TRM (F)	TNFR 1/2	Reduce NFκB / MAPK signalling	173-178
GM-CSF	TRM, F, EC	GM-CSFR	Reduce JAK STAT, PI3K, MAPK and NFκB signalling	179,180
<u>IL-17</u>	<u>TRM</u>	<u>IL-17R family</u>	<u>Reduce TRAF6, MAPK, TAK1 & NFκB signalling</u>	<u>181-183</u>
Kinase Inhibitors				
JAK inhibitors	F, TRM	JAK1 JAK2 JAK3 TYK2	Blockade of cytokine signalling via JAK STAT	145,146,149
SYK inhibitors	F, TRM	Fcγ receptor	Reduce IL-6 via MAPK / PKC	147,148
Fibroblast activation				
Cadherin-11 mAb	F	Cadherin-11	Reduce MAPK, NFκB, IL-6	153
Cyclin dependent kinase inhibitors (CDKi)	F	CDK1,2,4,6	Inhibit cell proliferation & survival, induce apoptosis	142,154,155
Epigenetic Modifier				
	F	DNA methylation Histone modification miRNA	Hypomethylation <u>LBH enhancer region</u> Increase H4 acetylation CXCL10 promoter Increase H4 acetylation IL-6 promoter Reduce miR-22 Reduce miR-20a Reduce miR203	<u>15,47,159</u> 44 184 185 186 187
Pro-resolving				
17-R RvD1		ALX, DRV1	Chondroprotective	164
Annexin A1	F, TRM, EC	ALX	Chondroprotective, increased TGFβ, prevent apoptosis	188
RvD3		ALX	Reduce leukocyte infiltration, prostaglandins, leukotrienes and thromboxane	163
15-epi-LXA ₄		ALX	Reduced STAT-1, IL-6,	

Comment [MOU2]: New references supporting therapies targeting IL-17 for the treatment of PsA

Comment [MOU3]: New references supporting therapies targeting DNA methylation

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Figures:**Figure 1. Embryological origins of mesenchymal tissues in the whole joint organ.**

To further understand the mechanisms and inter-relationships underpinning inflammation and tissue damage across the joint, it is important to consider the embryonic origins of joint tissues, which may shape the behaviour of these cell types in diseased adult tissues. Embryonic Mesoderm is the precursor for mesenchymal tissues comprising the ~~axial and~~ appendicular skeleton, synovium, cartilage, tendons, ligaments, joint capsule and their associated lymphatics and vasculature. Adult joint soft tissues are predominantly composed of cells of mesenchymal origin, including fibroblasts, endothelial cells and tissue resident macrophages (TRM). ~~The shared embryological origins of fibroblasts and endothelial cells shape the behavior of these cell types in diseased adult tissues in terms of their ability to exhibit activation and memory after exposure to inflammatory stimuli.~~ Yolk sac derived TRM are phenotypically genetically distinct from HSC derived lineages. TRM occupy subspecialized niches which dictate their heterogeneity and phenotype in adult tissues.

Figure 2. Molecular features of cells of mesenchymal origin in Rheumatoid pathological synovium.

Inset shows topographical location of cell types comprising RA synovium, consisting of lining and sublining layers. Synovial lining fibroblasts (blue) are CD34⁺CD90⁻, express PDPN, CD55 and release MMP-1 and MMP-13 implicated in tissue destruction. Fibroblast subsets concerned with proliferation, accumulation and inflammation ~~proliferation and inflammation~~ occupy the synovial sublining. ~~Proliferative~~ immunoregulatory fibroblasts (green) promote fibroblast accumulation and invasion. ~~These cells~~ express CD34 and release chemokines and cytokines generating gradients that promote leukocyte retention. Pathogenic fibroblasts (red) are a CD34⁺CD90⁺ subpopulation that highly express markers of ~~stromal~~ fibroblast activation and exhibit inflammation ~~stromal~~ memory. Pathogenic fibroblasts express TLR4 which mediates the damage sensing properties of these cells and downstream activation of the NFκB pathway via MAPK, JNK and JAK-STAT signalling pathways.

These phenotypic features sustain the pro-inflammatory pathogenic phenotype of this fibroblast subset. Fibroblasts in the synovial sublining are in close proximity to activated endothelial cells, expressing CD31, VCAM-1 and ICAM-1 and CD68⁺ tissue resident macrophages (TRM) which release pro-inflammatory mediators and proteases.

Figure 3: Mechanisms sustaining synovial inflammation, highlighting cross talk between cells of mesenchymal origin and leukocytes.

Cells of mesenchymal origin including fibroblast subsets, endothelial cells and tissue resident macrophages (TRM) are engaged in multidirectional cross talk, which sustains synovial inflammation. RA synovial fibroblasts promote leukocyte retention via release of cytokines and chemokine gradients and via contact with other cells of mesenchymal origin. Pro-inflammatory cytokines released by retained monocytes, T cells and TRM including IFN γ , TNF α and IL-1 β induce activated synovial fibroblasts to release high levels of PGE₂, GM-CSF and IL-6. TGF β released by TRM induces persistent expression of CXCR4 on synovial T cells, leading to their active CXCL12 mediated retention. RA synovial fibroblasts also release chemokines including CCL2, CCL4, CCL5, CCL8, CXCL8, CXCL12 and IFN β that promotes the recruitment, retention and survival of monocytes and CD4⁺ T cells. IL-6, TGF β 1 and VEGF released from TRM provide the necessary cues to promote an angiogenic environment required to sustain endothelial cell activation and dysfunction. The RA synovial fibroblast milieu further sustains an angiogenic environment through chemokine gradients comprising CXCL1-5 and CXCL8.