REVIEW

Pathogenic stromal cells as therapeutic targets in joint inflammation

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Abstract

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

Key points

- Joint inflammation and tissue damage are mediated by stromal cells of mesodermal origin
- Stromal activation and “memory” of previous inflammatory insults are shared mechanisms exhibited by fibroblasts, tissue resident macrophages and endothelial cells
- Recent advances characterising the phenotype and function of cells of mesenchymal origin highlight the distinct fibroblast subtypes mediating joint inflammation and tissue damage
- Mesenchymal stromal cell niches and their interactions with leukocytes are implicated in the persistence of joint inflammation
- To be effective, strategies to treat residual joint disease should target pathogenic stroma and associated immune cell cross talk

Introduction

Chronic inflammatory diseases affecting joint soft tissues include arthritis (synovium and cartilage), enthesopathy and tendinopathy. Collectively, these diseases comprise a significant global economic burden[1]. Each are characterized by inflammation of mesenchymal tissues that form the synovium, tendons, ligaments and joint capsule and in some cases structural damage to bone and cartilage. Inflammation of these tissues is broadly characterized by leuckocyte infiltration, fibroblast accumulation and neovascularization supporting cell expansion. In this article, we first review the pathophysiological basis of inflammation and tissue damage with respect to the embryological origins of joint mesenchymal tissues. We next discuss the stromal cell types populating joint mesenchymal tissues, including fibroblasts, tissue resident macrophages (TRM) and endothelial cells (vascular and lymphatic), highlighting their contribution and roles in chronic synovial inflammation and tissue damage. Finally, we discuss potential future therapeutic strategies to target inflammation across joint mesenchymal tissues that address the pathogenic stroma and associated immune cell cross talk.

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

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

The anatomical basis of inflammation and tissue damage relative to their embryological origin is summarized in Figure 1**.** Although parts of the axial skeleton derive from neural crest, mesoderm is the precursor for mesenchymal tissues

comprising the appendicular skeleton, synovium, cartilage, tendons, ligaments, joint capsule and their associated lymphatics and vasculature. These joint soft tissues are predominantly composed of cells of mesenchymal origin, including fibroblasts, vascular and lymphatic endothelial cells and TRM. The embryological origins of stromal cells may shape the behaviour of these cell types in diseased adult tissues. Notably, mesoderm derived fibroblast and endothelial cell populations both undergo sustained phenotypic changes after exposure to inflammatory stimuli, exhibiting stromal activation and a form of tissue 'memory'[2,3]. However, the distinct molecular markers expressed by these cell types vary, as we later discuss. TRM also exhibit complex activation states and "memory"[4]. The origins and renewal of TRM have been extensively reviewed and will not be repeated here [5-8]. The majority of TRM are established during embryonic development and persist into adulthood, rather than replacement from circulating adult monocytes [7,9-14]. During early gestation, macrophages are first observed and expand in the extraembryonic yolk sac during primitive hematopoiesis. Yolk sac derived hematopoietic stem cells (HSCs) emerge to form bone marrow precursor cells, which subsequently give rise to all immune cell lineages [7,15] (Figure 1). Importantly, yolk sac derived TRM are phenotypically distinct from HSC derived progeny [10]. The subspecialized adult tissue niches which TRM occupy dictate heterogeneity in the phenotype and functions of these cells in health and disease [16]. We next review how these mesenchymal cell populations are implicated in mediating inflammation and tissue damage in joint disease.

2.0 Cells of mesenchymal origin in the joint

In this section, we focus exclusively on cells of mesenchymal origin including fibroblasts, endothelial cells and TRM rather than on haematopoietically derived cells whose role in these processes (in particularly inflammation and damage) has been well documented[17-19]. We discuss the roles of these cells in normal joint physiology and their impact on inflammation and damage in joint disease. We highlight the recently identified mechanisms implicated in sustaining synovial inflammation, discussing the molecular features and pathological phenotypes of fibroblast subtypes, endothelial cells and TRM.

2.1 Fibroblasts and the healthy joint

The term ‘stroma’ was originally derived from the Greek word describing “a platform on which to lie” and is used to describe the supporting substance of tissue. Its principle role is to maintain the microenvironment required by the parenchyma; the important functional elements of each body system. The stroma comprises connective tissue, nerves, vessels and the extracellular matrices (ECM) and fluids which these cells produce[20]. Joint soft tissues including synovium, capsule, tendon and enthesis are predominantly composed of mesenchymal stromal cells. Fibroblasts are the most abundant cell type populating these joint connective issues[21] and synthesise the highly organized collagen rich scaffold necessary for joint structure and movement.

Fibroblasts are defined by their spindle shaped morphology, the absence of specific lineage markers of leukocytes, endothelium and epithelium and their ability to adhere to tissue culture plastic *in vitro*[22]. They are believed to arise from 3 distinct cellular origins: primary mesenchyme, local epithelial-mesenchymal transition (EMT) or bone marrow derived precursors (circulating fibrocytes)[23,24]. It is widely accepted that the majority of fibroblasts originate from primary mesenchymal cells and that fibroblasts can proliferate to generate new progeny[25,26]. In physiological conditions, fibroblasts provide mechanical strength to tissues by producing ECM components (type I, III and V collagen and fibronectin) as well as factors that regulate ECM turnover, including metalloproteinases (MMPs) and proteins involved in the formation of basement membranes (type IV collagen and laminin)[27,28]. Fibroblasts synthesise an array of paracrine factors[29] and exhibit mechanosensitive properties[30] to effect functional adaptation in normal joint physiology. The intimate relationship between fibroblasts and mesenchymal stromal cells (MSC) and the clinical use of MSC to repair damaged tissues has driven a renewed interest in fibroblasts as new therapeutic targets[21].

2.2 Mechanisms sustaining joint inflammation based on pathogenic stroma

2.2.1 Fibroblasts and the diseased joint

Traditionally, the diversity of stromal cells and in particular fibroblasts and their roles beyond those of space filling and ECM homeostasis have been underexplored in inflammation. Mesenchymal tissues in the joint including the synovium, enthesis and

tendons undergo phenotypic changes as a consequence of inflammation[31-33]. These include molecular and structural changes to the ECM, impacting upon the functional quality of the healed tissue[34]. Whilst it remains challenging to discern which is the initiating pathogeneic cell type, it is clear that stromal cells populating these tissues provide a niche conducive to sustaining chronic inflammation[2,35,36]. Recent work shows that fibroblasts vary phenotypically and functionally at different anatomical sites and contribute significantly to the identity of individual tissues, providing a so-called 'stromal postcode'[26]. Furthermore, it is known that, rather than acting as a bystander, fibroblasts are capable of actively participating and indeed orchestrating inflammation and immunity[36-38]. We next review how fibroblasts sustain inflammation, highlighting the mechanisms underpinning their activation, "memory" and phenotypic diversity, with particular focus on the synovial microenvironment.

Fibroblast activation and memory

Fibroblast activation is a recognized feature of diseases affecting the joint, whereby fibroblasts adopt a pro-inflammatory phenotype. This pathological feature has been identified in cancer [39], rheumatoid synovium[32,33] and tendon disease[31]. Fibroblast activation and memory therefore span both innate and adaptive immune responses, suggesting this is a highly conserved disease mechanism common to tissues of mesenchymal origin. There is now a growing list of cell surface molecules and secreted products which collectively provide a fibroblast activation marker "cassette". These include CD90 (Thy1), CD44, decay accelerating factor (CD55), VCAM-1 (CD106), uridine diphosphoglucose dehydrogenase, and prolyl-4-hydroxylase, Podoplanin (PDPN/gp38), endosialin (CD248) and Fibroblast Activation Protein (FAP) [31,36,37,40-42]. Fibroblast activation markers therefore represent important phenotypic alterations implicated in effecting the switch from resolving to persistent inflammation [42].

Epigenetic changes are implicated in fibroblast activation and memory. New insights into the epigenetics of inflammatory rheumatic diseases have been recently reviewed in detail elsewhere [43]. Prolonged exposure of RA synovial fibroblasts to TNFα reduce histone H4 levels and promote H4 acetylation [44]. This study showed that TNFα

removed the chromatin barrier from the CXCL10 promoter, permitting abundant binding of NF-κB family transcription factors and recruitment of transcriptional machinery[44]. DNA methylation is another important epigenetic modification identified in RA synovial fibroblasts occurring during the early stage of disease [45]. Further studies are required to identify the mechanisms underpinning DNA methylation and there appears to be important prognostic potential for differentially methylated genes as disease biomarkers [45]. The activated and aggressive phenotype of RA synovial fibroblasts is associated with global DNA hypomethylation[46]. Gaur *et al.* investigated if microRNAs moderate the methylation status of RA synovial fibroblasts, showing L-methionine increased DNA methylation compared to betaine [47]. Collectively these studies advance our understanding of how epigenetic changes are implicated in fibroblast activation and memory, informing future strategies to selectively target pathogenic fibroblasts.

Fibroblasts from different joint tissues maintain their phenotype, positional memory and topographic differentiation despite culture *ex vivo*. Fibroblasts isolated from RA synovium or diseased tendon exhibit stromal 'memory', whereby these cells show an enhanced subsequent capacity to respond to an additional inflammatory stimulus [2,31,44]. Therefore, sustained expression of activation markers by fibroblasts in the joint reflects their 'primed' status after exposure to an inflammatory stimulus. The processes underpinning innate memory have been extensively reported for leukocytes [48,49] and are gaining acceptance in tissue resident cells of mesenchymal origin. Engagement of TLR4 and downstream activation of the NFκB pathway is a prominent pathological feature of fibroblasts populating inflamed joint tissues [2,31,44]. These studies suggest that fibroblast memory is associated with altered NFκB responsiveness to an inflammatory stimulus [50]. Given the longevity of fibroblasts as tissue resident cells and the relatively low rates of cell turnover in the joint [51], the effects of stromal memory in tissues such as synovium and tendon are likely to be long lived. In contrast, dermal fibroblasts show higher rates of turnover and do not exhibit memory, suggesting this process of stromal memory may vary according to anatomical location [2,52,53]. Rheumatic diseases follow a characteristic anatomical pattern of joint and organ involvement. Mechanisms regulating the predilection of specific joints for developing particular forms of arthritis (for example osteoarthritis

(OA) compared to rheumatoid arthritis (RA)) have been reviewed in detail [54]. These include site-specific local cell types driving disease, systemic triggers affecting local cell types and site-specific exogenous factors activating cells locally. Therefore the mechanisms underpinning activation of stromal cells depends on the local anatomical tissue niche[54].

Fibroblast diversity

Recent work shows that tissue resident fibroblasts help define the pattern of joints involved in RA [55,56]. The concept of epigenetically-driven anatomical diversity of synovial fibroblasts provides an attractive mechanism to explain the clinical observations that different types of arthritis affect distinct types of joints. For example, OA and psoriatic arthritis often involve the distal interphalangeal joints, whereas RA is frequently symmetrical and more commonly affects the MCP joints. In contrast, ankylosing spondylitis (AS) mainly targets spinal ligaments and entheseal tissue[57]. Such studies have prompted improved characterization of the phenotypes of fibroblast subsets and their different proposed roles. In RA, synovial fibroblasts undergo distinct changes in function, including loss of immunosuppressive response in early disease, followed by later acquisition of an immuno-stimulatory phenotype[58]. Highly conserved homeobox (HOX) transcription factors specify regional identities of cells and tissues throughout the body [59,60] and adult fibroblasts retain key features of embryonic positional HOX gene expression [56]. Fibroblasts also vary according to their anatomical location in relation to tissue structures at an individual site and the exogenous stimuli which they receive [54,56,61]. Whether variability can be attributed to the plasticity of individual fibroblasts necessary for responding to different environmental cues and whether phenotypic variation can be used to define distinct subsets of fibroblasts specialized for different niches remains unclear.

The synovium is composed of lining and sub-lining layers of fibroblasts which vary in terms of phenotype and function. Single cell RNA sequencing and immunohistochemistry have revealed that RA synovial fibroblasts can be broadly characterized into 3 subsets, highlighted in Figure 2. Synovial lining fibroblasts are $CD34^{-}CD90^{-}CD55^{+}$ and Cadherin 11^{+}. This lining subset synthesizes MMP-1 and MMP-3 which mediate tissue damage in the inflamed joint [62]. Fibroblasts populating the synovial sublining are predominantly comprised of 2 populations. $CD34^{+}CD90^{-}$

fibroblasts release CXCL12, CCL2 and IL-6 and drive fibroblast accumulation and invasion. A second population of $CD34^{-}CD90^{+}$ fibroblasts with a pro-inflammatory phenotype express markers of fibroblast activation [62,63]. These fibroblast subsets between them degrade articular cartilage, mediate stromal memory, sense tissue damage via TLR4 activation and have altered responsiveness to signalling pathways converging on NFκB responsiveness[26,33,50,62] (Figure 2). Having highlighted the complexity of discrete synovial fibroblast subtypes, we next discuss the phenotypes and functions of other mesenchymal cell types including endothelial cells and TRM and their respective roles in joint disease.

2.2.2 The endolymphatic niche in the diseased joint

Other mesenchymal stromal tissues including the vasculature and lymphatics contribute to sustaining inflammation across the joint organ. Neo-angiogenesis is a prominent feature of disease of mesenchymal joint tissues and impacts upon changes in tissue architecture and pain perception[64]. In health, vascular endothelial cells regulate blood flow, vessel wall permeability and leukocyte extravasation into tissues, regulating the inflammatory process [65-68]. In lymph nodes and tertiary lymphoid tissues, high endothelial vessels (HEVs) provide specialized microenvironments for efficient entry of lymphocytes into tissues in an L-selectin dependent process[69]. The phenotypes of endothelial cells change as inflammation transitions from acute to chronic and also between activation of innate and adaptive immune systems [67]. Endothelial cell phenotypes are poorly characterized in tendon and entheseal tissues. However, in RA synovium, these cells have been described as activated, angiogenic, apoptotic and leaky, a process found in many tumour microenvironments [70]. During prolonged exposure to inflammatory stimuli endothelial cells become activated, exhibit memory and express adhesion molecules including ICAM, VCAM-1 and CD31 (PECAM-1) [3,71-73] (Figure 2). These activated endothelial cells also present chemokines and initiate leukocyte migration from blood to local tissues[70]. Endothelial activation is a cause and consequence of endothelial dysfunction[74,75], culminating in increased microvascular permeability, extravasation of plasma and joint oedema. Release of angiogenic factors including VEGF triggers angiogenesis, provide necessary nutrients and oxygen to meet the metabolic demands of the inflamed tissue.

Importantly, neo-angiogenesis further promotes the retention and survival of immune cells at inflamed sites, thereby sustaining chronic inflammation [38]. These angiogenic processes occur during normal inflammatory immune responses (i.e vaccination)[76], however whether angiogenesis that occurs in joint disease is a cause or effect of pathology remains unclear.

Stromal lymphatic vessels form a one-way conduit for tissue fluid and leukocytes in health and disease [77]. During adaptive immune responses, antigen presenting cells travel to lymph nodes via lymphatic vessels, which highly express PDPN, implicated in fibroblast activation[78]. The permeability of lymphatic vessels is a tightly regulated dynamic process that alters during health and disease [79]. Lymphatic vessel growth (lymphangiogenesis) is a primary response during acute inflammation, which becomes dysregulated in chronically inflamed adult tissues[80]. In experimental murine models of inflammatory arthritis, lymphatic vessels and nodes draining the diseased joint undergo an initial expansion phase to expedite lymphatic clearance. This expansion phase is followed by a collapsed phase, characterized by structural damage to lymphatic vessels and reduced lymphatic clearance [79,81]. Studies demonstrate alteration in lymphatic vessel function and lymph node volume also occur in patients with RA flare [82]. Therapies targeting aberrant lymphatic function have shown promise in preclinical models of inflammatory arthritis and may prove efficacious in RA [79].

2.2.3 Tissue Resident Macrophages in the diseased joint

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

In inflamed synovium, TRM mediate immune surveillance through expression of a variety of pattern recognition receptors, notably Toll-like receptors (TLR) TLR2 and TLR4 and facilitate the recruitment of infiltrating leukocytes, incuding monocyte

derived macrophages [85-87]. TRM induce joint inflammation through release of TNFα, IL-1β IL-6, GM-CSF and PGE_2, driving fibroblast accumulation, angiogenesis, leukocyte recruitment and tissue damage via protease secretion (Figure 2). The essential role of non-classical Ly6C-monocytes has been reported in murine arthritis models [88]. This study highlights the phenotypic heterogeneity of synovial TRM, demonstrating how macrophage activation status regulates disease progression and resolution. In support of this, human RA synovial macrophages exhibit distinct transcriptional profiles associated with disease activity and therapy [89]. However, distinction between TRM and infiltrating macrophages is currently hampered by a lack of specific markers that distinguish between these populations in diseased human tissues.

The pro-inflammatory milieu in the inflammed synovium triggers an active process of lipid mediator class switching and the subsequent release of families of specialized proresolving mediators (SPM). These include lipoxins, resolvins, protectins and maresins, that are generated via transcellular biosynthesis and are concerned with mediating resolution of inflammation[90-94]. These bioactive lipid mediators initiate programmes which halt neutrophil infiltration, potentiate monocyte recruitment, moderate vascular permeability and promote phagocytosis and drainage of apoptotic cells[95]. The mechanisms mediating resolution in inflammatory arthritis have been reviewed in detail and are not covered here [96]. TRM are key regulators of repair and fibrosis across all tissue types [34] and are also implicated in mediating resolution of inflammation. Distinct populations of resolution phase macrophages have been identified in systemic murine inflammation models that express Alox15, Timd4 and Tgfb2, which terminate leukocyte recruitment and promote clearance [97]. However, the precise phenotypes of TRM mediating resolution in human joint disease requires further investigation.

2.2.4 Cross talk between cells of mesenchymal origin

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

RA synovial fibroblasts act as sentinel cells that can "sense" tissue damage. This occurs via the binding of damage associated molecular patterns (DAMPs) including HMGB1, heat shock and S100 proteins[100,101]. Tenascin-C a matrix protein induced upon tissue damage also activates TLR4 mediated sterile inflammation [102]. Binding of these ligands to TLR4 induces a high alert state, favouring the development of chronic inflammation [50,103]. Engagement of TLR4 activates Myd88 signalling pathways, inducing pro-inflammatory cytokine release via NFκB activation [48]. Consequently, activated synovial fibroblasts are primed to release a broad range of pro-inflammatory mediators. These localised cytokine and chemokine gradients promote the migration, retention and survival of leukocytes and TRM,[42,104] creating a complex functional syncytium conducive to sustaining inflammation, highlighted in Figure 3. The processes mediating leukocyte trafficking between stromal compartments in RA are recently reviewed in detail elsewhere [105].

Fibroblast – immune cell cross talk

RA synovial fibroblasts promote leukocyte retention via release of cytokines and chemokines and via contact with other cells of mesenchymal origin. Pro-inflammatory cytokines released by retained monocytes, T cells and TRM including IFNγ, TNFα and IL-1β induce activated synovial fibroblasts to release high levels of PGE_2, GM-CSF, IL-6. These cytokines exert differing effects on leukocyte activation. PGE_2 moderates chemokine production and promotes Th2, Th17 and Treg responses [106]. IL-6 drives CD4+ T cells towards Th17 activation [107], whereas GM-CSF promotes neutrophil survival and monocyte differentiation in the inflamed synovium [26,108]. Nguyen *et al*. demonstrated that IL-6 and other inflammatory cytokines and chemokines are regulated by a positive feedback loop that selectively operates in fibroblasts involving

leukemia inhibitory factor (LIF), LIF receptor and STAT4 [109]. TGFβ, also found at high levels in RA synovium induces persistent expression of CXCR4 on synovial T cells, leading to their active CXCL12 mediated retention, providing an additional mechanism for immune cell retention [110]. RA synovial fibroblasts also release a repertoire of chemokines, generating a gradient consisting of CCL2, CCL4, CCL5, CCL8, CXCL8, CXCL12 and IFNβ [26,111,112]. This chemokine gradient actively promotes the recruitment, retention and survival of monocytes and CD4+ T cells at the inflamed synovial site (Figure 3). CXCL12, VCAM-1 (CD106) and IL-6 therefore constitute part of a 'stromal address code', critical for leukocyte survival and differentiation [26].

Endothelial cell cross talk

Resident stromal cells populating inflamed synovium modulate the ability of endothelial cells to recruit leukocytes via release of soluble mediators or direct cell-cell contact. Fibroblasts isolated from healthy patients are known to regulate the cytokine-sensitivity of vascular endothelium, while fibroblasts associated with chronic inflammation adopt a pro-inflammatory phenotype [29,113]. Cytokine and chemokine gradients mediate and sustain cross talk between endothelial cell, synovial fibroblast and TRM populations. IL-6, TGFβ1 and VEGF released from TRM provide the necessary cues to promote an angiogenic environment required to sustain endothelial cell activation and dysfunction (Figure 3). This is supported by antibody neutralisation of IL-6, which diminished the ability of endothelial cells to bind lymphocytes in co-cultures with RA fibroblasts [29].

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

2.3 Mesenchymal cells and their role in joint damage

In health, early damage repair mechanisms maintain the integrity of joint soft tissues. In joint disease, sustained inflammation, tissue remodeling and fibrosis ensue, resulting in irreversible tissue damage. We next discuss how cells of mesenchymal origin mediate fetal scarless healing and highlight the mechanisms by which they induce damage across adult joint tissues.

In contrast to normal adult tissues, early human and murine fetal wounds and wounds in Nude (FoxN1 deficient) mice heal without scar formation[114]. Fetal wounds show diminished numbers of immune cells and lower levels of cytokines compared to adult tissues [115-118]. Differences between embryonic and adult tissue healing are also attributed to the milieu of pro-fibrotic growth factors released by TRM, including those of the TGFβ family. TGFβ1 levels are reduced and this growth factor shows accelerated clearance in embryonic compared to adult tissue repair [119-121]. Collectively these studies indicate a role for immune cell derived cytokines including TNFα and TGFβ in tissue scarring and healing[122]. Other studies highlight differences between fetal and adult fibroblasts and localized production of MMP-9 and MMP-13 in the scarring process [114]. Fetal fibroblasts show enhanced synthetic function, increased rate of turnover of collagen, hyaluronic acid, ECM components and increased migration velocity compared to adult fibroblasts, suggesting rapid healing may also play a role in scarless tissue repair[123-125].

In adult tissues, fibroblasts and TRM directly contribute to joint destruction, bony erosions and remodeling through expression of enzymes such as MMPs[126]. MMP-2, MMP-9 and MMP-13 have been specifically implicated in the pathogeneis of RA and OA[127]. MMP-9 is also upregulated by CXCL12 (SDF-1) a key chemokine secreted by synovial fibroblasts[128]. FAP is highly expressed within RA synovium and co-localises with MMP-13, where it appears to play a role in tissue degradation[129]. Cathepsins, a major group of proteases involved in joint remodeling are also upregulated in the diseased joint[130]. Additionally fibroblasts can indirectly contribute through cross talk with TRM and lymphocytes, further amplifying processes driving tissue damage (Figure 3), whilst also presenting antigen to tissue infiltrating lymphoctyes[131].

Pathological conditions in which cells of mesenchymal origin play a role include chronic inflammation (e.g. RA, chronic skin wound healing), tissue fibrosis (e.g. COPD) and cancer (e.g. breast cancer). Interestingly, while these diseases differ dramatically in aetiology and genetic predispositions, they converge in terms of phenotype and function of the stromal component. Fibroblasts expand in the RA synovial tissue and in the tumor parenchyma, while fibrosis is characterized by profound changes in myofibroblast phenotype and function across different organs such as the lungs and kidneys [132]. Whether these fibroblast properties are intrinsic phenotypic changes acquired as a consequence of exposure to chronic inflammation, or are derived from the conditioning of the pathogenic infiltrating cells is still under investigation and seems to differ in the different conditions[37]. Lafevre *et al* reported epigenetically programmed aggressive cells may "spread" arthritis from inflamed to uninflamed joints in the early stages of disease, [133]. PDPN expressing lining synovial fibroblasts are migratory and mediate release of cartilage destructive MMPs [33,62]. Collectively, these data raise the possibility of distinct mesenchymal cell subsets implicated in mediating the effects of tissue damage in the diseased joint. We next discuss how the possibility of selectively targeting pathogenic stromal subpopulations mediating inflammation and tissue damage informs the development of future strategies to successfully treat joint disease.

3.0 Shaping the future landscape: therapeutic targeting of mesenchymal cells

Cells of mesenchymal origin including fibroblasts, TRM and endothelial cells constitute the major cell types populating joint soft tissues. We have discussed the roles and mechanisms by which these cells mediate joint inflammation, highlighting their ability to act as immune sentinel cells, their capacity for activation, positional memory and their altered phenotypes comprising multiple cellular sub-populations. Multidirectional cross talk between stromal cell populations further fuels the development of persistent inflammation. Given these important roles and associated biological complexities, it is likely that residual disease activity in patients treated with immune therapies may be attributable to stromal mediated inflammatory responses, which are refractory to

current therapies that target immune cell populations [134]. New therapeutic approaches are therefore required to 'break the cycle and reset the system', particularly in scenarios where inflammation becomes independent of the inciting stimulus. Given the limited capacity of joint tissues to regenerate once damaged, there are significant challenges associated with curbing tissue damage, which might be accomplished through moderating persistent inflammation as a driver of fibrosis. We next discuss the requirement for future strategies to address the pathobiology concerned with the stromal microenvironment, targeting cells of mesenchymal origin. We review the drug classes in current clinical use, those in early phase clinical trials and strategies with pre-clinical potential to target stromal mediated joint disease. The cellular and molecular targets and the mechanism of action through which these drug classes function are summarized in Table 1.

Existing licensed therapies

Nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids provide symptomtic relief for a broad array of conditions targeting inflammation and pain. Their clinical use in the management of a multitude of diseases affecting the joint is well established [135-138]. These therapies target fibroblasts, TRM and endothelial cells via differing biological modes of action. Inhibition of COX activity by NSAIDs dampens release of prostaglandins, leukotrienes and thromboxane A_2. Corticosteroids act via the glucocorticoid receptor to inhibit $cPLA_2$, regulate expression of NFκB / MAPK target genes and dampen release of inflammation initiating eicosanoids. Whilst NSAIDs and corticosteroids continue to provide background anti-inflammatory therapy for many rheumatic diseases, they are both associated with well documented adverse systemic effects. Importantly, COX-2 selective NSAIDs also dampen protective endogenous resolution responses [139,140], which may paradoxically impede the capacity of inflamed joint tissues to heal.

Monoclonal antibodies enable precise molecular targeting of cytokines mediating joint inflammation. The biological modes of action and efficacy of therapeutic inhibitors of IL-1, IL-6, TNFα and IL-17 in current clinical use are well reported and listed in Table 1. One disadvantage associated with selective cytokine inhibition is the failure of this

approach to fully target stromal mediated inflammatory responses and address the complex multidirectional cross talk between mesenchymal cell populations. Similarly targeting chemokine gradients is an attractive strategy to moderate leukocyte retention [141]. However chemokine antagonists including AMD3100 targeting CXCR4 are associated with adverse systemic effects [142] and the plethora of chemokines mediating stromal inflammatory responses presents a further therapeutic challenge.

Therapies in early phase clinical trials

GM-CSF, predominantly produced by activated T cells, monocytes and macrophages is also released by tissue resident cells of mesenchymal origin [143]. Humanised IgG1 monoclonal antibodies to GM-CSF prevent interaction of this cytokine with its receptor, reducing downstream signalling pathways converging on NFκB. GM-CSF has shown potential as a therapeutic target in autoimmune and inflammatory disorders, including RA. Therapies targeting GM-CSF or its receptor have shown encouraging results in more recent pre-clinical studies and are reviewed in detail elsewhere [143]. Recent phase IIb studies have demonstrated that long term mavrilimumab treatment maintained clinical responses and was well tolerated in RA patients with inadequate response to DMARD's[144]. Further investigation is required to determine the efficacy of GM-CSF targeted therapies to modulate stromal mediated inflammatory responses in the joint.

Kinase inhibitors targeting JAK and SYK signalling pathways have been investigated for their therapeutic utility to reduce cytokine release through JAK STAT [145,146] or MAPK / PKC [147,148] blockade respectively (Table 1). Baricitinib, an oral reversible inhibitor of JAK1 and JAK2 has shown therapeutic value in RA patients. This treatment was associated with significant clinical improvements in patients with an inadequate response to methotrexate compared with placebo and adalimumab treated groups [149]. Protein kinase inhibitors target a broad range of cells types with reported off target effects, highlighting the importance of understanding the pharmacology of these drugs beyond the kinome [150].

Potential future strategies to target pathogenic stroma

Developments in cancer medicine targeting cancer associated fibroblasts populating tumour stroma have informed potential future strategies to target pathogenic stroma in rheumatic disease [151,152]. Targeting pathogenic stroma presents a considerable therapeutic challenge due to the biological complexity underpinning activation, memory and phenotypic diversity exhibited by these mesenchymal cell populations. Potential future strategies to treat residual rheumatic disease might include targeting activated fibroblast subtypes, use of epigenetic modifiers or resolution agonists to target stromal mediated inflammation. Pre-clinical evidence supporting these approaches are discussed below.

Selective targeting of distinct fibroblast subtypes mediating joint inflammation and tissue damage is a potential therapeutic strategy to target pathogenic stroma. Cadherin-11 is known to regulate synovial fibroblast inflammation, synergizing with IL-1β and TNFα to regulate IL-6 release [153]. This study showed that cad-11 deficient mice or anti–cad-11 mAb therapies reduced inflammation in arthritic mice, suggesting that cadherin expression regulates the inflammatory capacity of synovial fibroblasts. Cyclin dependent kinases regulate cell proliferation and survival via specific inhibitors (CDKi) and are potential therapies to target fibroblast accumulation in RA synovium (Table 1). CDK pathways become dysregulated in cancer, leading to the development of anti-cancer drugs including the CDKi Roscovitine [154]. In synovial fibroblasts, IL-6 and MMP-1 are known to be regulated by CDKi p21 [155]. Given that $CD34^{+}CD90^{-}$ 'immunoregulatory' fibroblasts are highly proliferative, invasive and produce IL-6[62], CDKi therapies are a potential strategy to target this fibrolast subset mediating joint disease.

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

The roles of proresolving mediators in joint health and disease are increasingly understood, identifying resolution agonists as potential therapies to moderate joint inflammation and promote tissue repair [96]. The biological modes of action of proresolving mediators or 'immunoresolvents' are well established from *in vitro* and *in vivo* studies and include limiting PMN infiltration, stimulating efferocytosis and activation of endogenous tissue protective mechanisms [90-93,157,158]. Whilst immunoresolvents target leukocytes, their biological actions are not associated with immunosuppression [83,159]. Importantly, proresolving mediators also target fibroblasts, TRM and endothelial cells types [160-162] and therefore possess the capacity to modulate stromal mediated inflammatory responses across joint tissues. Approaches to potentiate resolution processes include dietary supplementation with proresolving precursors, blocking catabolism of proresolving mediators or local delivery of stable analogues binding proresolving receptors [96]. The pro-resolving mediator RvD3 was found to limit leukocyte infiltration and paw joint eicosanoid levels in murine inflammatory arthritis [163]. The stable epimer 17R-RvD1 significantly attenuated arthritis severity, cachexia, paw oedema, leukocyte infiltration and shortened the remission interval, showing cartilage protective actions in murine models of acute inflammatory arthritis[164]. *In vitro* studies also highlight the capacity of 15-epi-LXA_4 and MaR1 stable epimers to regulate PDPN, STAT-1 and IL-6 in IL-1β stimulated diseased human tendon stromal cells [35,165]. Collectively these studies suggest resolution pharmacology may be an important future therapeutic tool to address stromal pathobiology in the joint.

Conclusions

Stromal cells of mesenchymal origin including fibroblasts, tissue resident macrophages and endothelial cells are pivotal populations regulating health and disease in musculoskeletal tissues. New insights are beginning to reveal the mechanisms underpinning the activation and dysfunction of mesenchymal stromal cells and their contribution to sustaining chronic joint inflammation. The discovery that distinct synovial fibroblast subsets mediate joint inflammation and damage will inform precision therapeutic targeting of pathogenic stromal cell populations. These

discoveries shape the future therapeutic landscape, presenting exciting new approaches to address the pathogenic stromal microenvironment. Harnessing the capacity to modulate cross talk between leukocyte and pathogenic stromal cell populations is a critical barrier to overcome in our quest to advance therapeutic strategies for patients with refractory joint disease.

Glossary of terms

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

Mesenchymal: Embryonic connective tissue derived from the mesoderm

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

Stromal cell: Non-haematopoietic, tissue resident cells.

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

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

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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
IL-17	TRM	IL-17R family	Reduce TRAF6, MAPK, TAK1 & NFκB signalling	181-183
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, NFkB, 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 Increase H4 acetylation CXCL10 promoter Increase H4 acetylation IL-6 promoter Reduce miR-22 Reduce miR-20a Reduce miR203	45,47,156 44 184 185 186 187
Pro-resolving				
17-R RvD1	F, TRM, EC	ALX, DRV1	Chondroprotective	164
Annexin A1		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, Podoplanin	35,165

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. Mesoderm is the precursor for mesenchymal tissues comprising the 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). Yolk sac derived TRM are phenotypically 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 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 occupy the synovial sublining. 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 fibroblast activation and exhibit inflammation 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 IFNγ, TNFα and IL-1β induce activated synovial fibroblasts to release high levels of PGE_2, GM-CSF and IL-6. TGFβ 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 IFNβ that promotes the recruitment, retention and survival of monocytes and CD4+ T cells. IL-6, TGFβ1 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.