

Stroma

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Stroma: Fertile soil for inflammation

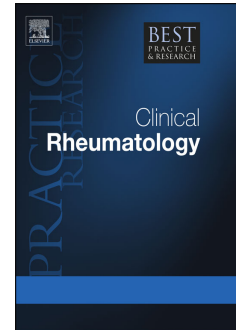
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Stroma: Fertile soil for inflammation

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Inflammation, fibroblast, stroma, stromal cell, rheumatoid arthritis, synovium

Contents

Abstract	Error! Bookmark not defined.
1.0 Introduction	Error! Bookmark not defined.
2.0 The stroma.....	Error! Bookmark not defined.
2.1 Fibroblast biology	Error! Bookmark not defined.
2.2 Pathogenic Stroma.....	Error! Bookmark not defined.
2.3 Acute inflammation	Error! Bookmark not defined.
2.4 The recruitment postcode	Error! Bookmark not defined.
2.5 Fibroblast activation	Error! Bookmark not defined.
3.0 The stroma in chronic inflammation	Error! Bookmark not defined.
3.1 Accumulation of epigenetic changes and stability of the inflamed microenvironment.....	Error! Bookmark not defined.
3.2 Recruitment of leukocytes to sites of chronic inflammation.....	Error! Bookmark not defined.
3.3 Retention of lymphocytes.....	7
3.4 Tertiary lymphoid organs (TLOs).....	Error! Bookmark not defined.
3.5 Inhibition of leukocyte apoptosis	Error! Bookmark not defined.
3.6 Tissue destruction in RA	Error! Bookmark not defined.
3.7 p21 cyclin dependent kinase inhibitors (CDKi)	8
3.8 The synovium in patients with early rheumatoid arthritis	9
4.0 Biological agents in RA that target the stroma	9
4.1 Possible future stromal targets.....	10
5.0 Conclusions.....	10
6.0 Practice points	11
7.0 Research Agenda	11

Abstract

Biological therapies for the management of immune mediated inflammatory diseases such as rheumatoid arthritis have proven to be extremely successful in recent years. Despite these successes, even the most effective of therapies do not lead to cure. Why chronic inflammation persists indefinitely within the rheumatoid synovium despite an absence of continuous stimulation, and why some patients with early synovitis progress to persistent disease whilst others do not, has remained unexplained. In contrast to the paradigm that stromal cells are biochemically active but immunologically passive, there is now growing evidence that stromal components from the rheumatoid synovium play a crucial part in the immunopathology of rheumatoid arthritis. Stromal cells play a central role in the transformation of an acute, resolving to a chronic inflammatory process, and to the persistence of synovial inflammation and joint destruction through a variety of immune mechanisms. Therapeutic manipulation of the stroma is a largely unexplored, yet potentially vital area of research. Targeting pathogenic stromal cells has the potential to provide a cure for chronic inflammatory disorders such as rheumatoid arthritis.

1.0 Introduction

Inflammation is an intrinsic, biological response that occurs to protect organisms against harmful stimuli. At a cellular level, inflammation is characterised by an influx of leukocytes into damaged or infected tissue. The duration, type and magnitude of leukocyte influx is controlled by cytokine and chemokine gradients. For an inflammatory response to resolve completely, these gradients must be removed, and leukocytes must be cleared from the site of pathology, either by migration out of the site, or via apoptosis (1).

Episodes of acute inflammation most often occur in the context of infection; these episodes are transient, often self-limiting and are followed by a phase of tissue repair leading to health. Chronic, persistent inflammation in contrast often occurs in the absence of a pathogen, and in the case of autoimmune diseases such as rheumatoid arthritis, may persist indefinitely even in the absence of a harmful external stimuli.

The current and established pharmacological management of most autoimmune pathologies aims to dampen the persistent, harmful inflammatory response in the tissue affected. This approach of minimising damage and progression of disease is however suboptimal. The optimal approach is to reverse the fundamental pathological process.

Our understanding of the role that stromal cells play in inflammation has changed considerably. Stromal cells, in particular fibroblasts, are emerging as key drivers in the genesis of tissue specific inflammation, modulation of tissue microenvironments, transformation from acute to chronic inflammation and persistence of inflammation through a multitude of immune-mediated modalities, particularly in rheumatoid arthritis (2). These discoveries raise the possibility that stromal cells may turn out to be novel therapeutic targets in the discovery of a cure for chronic inflammatory diseases.

This review aims to highlight the role that fibroblasts, a key cellular component of the stroma, play in the genesis and persistence of chronic inflammation in rheumatoid arthritis and to provide an insight into how currently available biologic agents modulate stromal cell function. It will also outline possibilities for novel stromal cell targets, which may be beneficial in future therapies for rheumatoid arthritis.

2.0 The stroma

Tissue stroma comprises the subendothelial basement membrane and the underlying connective tissue containing stromal cells such as pericytes, fibroblasts and organ specific stromal cells (astrocytes, hepatocytes) and epithelial cells as well as adipocytes (2). The extracellular matrix that lies between stromal cells is comprised mainly of glycosaminoglycans and proteoglycans. Lymphatic and blood vessels and nervous tissue is interspersed throughout the stroma. It is through this matrix that leukocytes migrate to and from an area of inflammation (3). Considerable effort has been expended in targeting vascular endothelial cells in cancer and inflammation. While new anti-angiogenic agents have shown promise in some cancers, their application to autoimmune diseases remain disappointing

2.1 Fibroblast biology

The fibroblast is the most common cell type within the stroma (4). Fibroblasts are identified by their spindle-shape morphology, expression of interstitial collagens (types I and III) and their adherence to plastic in culture (5). Fibroblasts express numerous markers which aid in their identification (6) (Table 1). However None of these markers is highly specific to fibroblasts, nor is any marker reliably present in all fibroblasts (7). Because of this, there has been until recently a lack of information on the origin, function and role of fibroblasts in disease (8).

Mesenchymal Stromal cells (MSCs) are multipotent cells, which are currently undergoing extensive investigation for use in tissue repair. They share common cell surface markers, morphology and immunologic properties with fibroblasts (9). It is currently, although not exclusively accepted,

that MSCs and fibroblasts are distinct cell types, set apart by the ability of MSCs to differentiate into any mesodermal tissue (10) and the discovery that fibroblasts are not always mesodermal in origin. For example fibroblasts in the head and neck arise from the ectoderm through a process termed epithelial to mesenchymal transition (EMT) (11).

Under physiological conditions, the stroma functions primarily to provide structure by synthesising and remodelling the extracellular matrix (ECM). Fibroblasts synthesise type I, type III and type V collagen, and fibronectin (12) but also have a role ECM degradation via matrix metalloproteinases (MMPs) (13). Thus fibroblasts have prime responsibility for the maintenance of ECM homeostasis by regulating matrix turnover (7). Fibroblasts also help in maintaining biochemical homeostasis between adjacent epithelial cells (7) and have a key role in tissue development, differentiation and repair (including fibrosis) (14).

Of the cells that comprise the stroma, the fibroblast has been most implicated in generating and directing both the innate and adaptive immune response in health and disease (14). Synovial fibroblasts from patients with rheumatoid arthritis have provided key information on how the stroma is responsible for persistence of chronic inflammation. This is discussed in detail below.

Table 1: Fibroblast markers

Marker	Function	Fibroblast subtype	Expressed elsewhere
Vimentin	Intermediate filament associated protein	Miscellaneous	Endothelial cells, myoepithelial cells, neurons
α -SMA	Intermediate filament associated protein	Myofibroblasts	Vascular smooth muscle cells, pericytes, myoepithelial cells
Desmin	Intermediate filament associated protein	Skin fibroblasts	Muscle cells, vascular smooth muscle cells
FSP1	Intermediate filament associated protein	Miscellaneous	Invasive carcinoma cells
Discoidin-domain receptor 2	Collagen receptor	Cardiac fibroblasts	Endothelial cells
FAP*	Serine protease	Activated fibroblasts	Activated melanocytes
α 1 β 1 integrin	Collagen receptor	Miscellaneous	Monocytes, endothelial cells
Prolyl 4-hydroxylase	Collagen biosynthesis	Miscellaneous	Endothelial cells, cancer cells, epithelial cells
Pro-collagen1 α 2	Collagen-1 biosynthesis	Miscellaneous	Osteoblasts, chondroblasts
CD248*	Unknown	Miscellaneous	Pericytes
VCAM-1	Cell adhesion	Miscellaneous	Activated endothelial cells
gp38*	Invasiveness in cancer	Miscellaneous	Alveolar cells, podocytes, endothelial cells, neurons
Cadherin-11*	Homotypic adhesion	Miscellaneous	Nil

Adapted from Kalluri and Zeisberg (2006) (7) and Filer (2013) (15) *of particular significance as a possible therapeutic target

2.2 Pathogenic Stroma

Stromal tissue has been shown to play a functional role in three chronic pathological states: malignant disease, tissue fibrosis and chronic inflammation (16). In all of these conditions, stromal cells have been observed to undergo specific, disease dependent phenotypic and functional changes which promote the disease state (17). In malignancy for example, in addition to production of the

non-tumour extracellular matrix that facilitates growth of solid tumours, the stroma has been shown to be a key driver of tumour progression by inhibiting apoptosis of malignant cells in breast carcinoma (18). Fibroblast activation protein (FAP), a serine protease that is selectively expressed in reactive fibroblasts of epithelial cancers has also been noted to have a pathogenic role in malignancy (19). FAP blockade in mice models of pancreatic ductal carcinoma has been shown to induce hypoxic necrosis of tumour and stromal cells in a process that is dependent on Interferon gamma (IFN γ) and TNF alpha (TNF α) (20). In addition to these effects, reactive stroma and cancer associated fibroblasts are also known to facilitate tumourigenesis and metastasis via generation of oncogenic signals (21) and promotion of angiogenesis (22).

2.3 Acute inflammation

During episodes of acute inflammation, vascular endothelial cells (ECs) are responsible for the immediate recruitment of specific leucocyte subsets to an area of acute pathology by expressing specific adhesion molecules and chemokines in response to biochemical signals generated by inflammatory/infectious/traumatic tissue injury (23-25). In the acute inflammatory response to infection, tissue resident macrophages may release cytokines such as IL-1 or TNF α . Along with bacterial components, and activated complement fragments, these cytokines stimulate vascular ECs to express adhesion molecules that interact with surface presented chemokines or other mediators, that, in turn, activate leukocyte integrins (2). The types and patterns of adhesion receptors expressed as well as the chemokine gradients are determined by the nature of the stimulus driving the inflammatory response (26-30). For example, $\alpha_M\beta_2$ -integrin binds intracellular adhesion molecule 1 (ICAM-1) to stabilize adhesion and supports migration of neutrophils to the site of acute inflammation (2). Capturing peripheral blood leukocytes (PBLs) to an area of chronic inflammation in contrast, requires $\alpha_4\beta_1$ -integrin to bind to vascular cell adhesion molecule 1 (VCAM-1), a process which is stabilised by the chemokine CXCR3 (27).

Whilst the EC is the principal cell type controlling leukocyte migration into an area of acute inflammation, stromal components are known to have an important role in the regulation of this response. When co-cultured ECs and fibroblasts are exposed to the pro-inflammatory cytokines TNF α and IFN γ , fibroblasts reduce lymphocyte migration in an IL-6 dependent fashion; these effects are not seen in the absence of TNF α /IFN γ (31), suggesting that healthy fibroblasts play an immunomodulatory role by preventing inappropriate recruitment of leukocytes to areas of acute inflammation. This is in contrast to the pathogenic role that fibroblasts play in chronic inflammation

2.4 The recruitment postcode

The specific combination of receptors, chemokines and other agents driving leukocyte transmigration is often referred to as the recruitment postcode (2). When fibroblasts and ECs are co-cultured, alterations in genotype are observed in both cells. These changes are dependent on the anatomical source of the fibroblasts (32). The phenotype of the fibroblast itself however, is also altered by inflammatory stimuli (8, 33). It is therefore probable that the fibroblast, rather than the endothelial cell is the primary protagonist in modulating tissue-specific, stimulus-specific inflammatory responses through control of endothelial cell function.

2.5 Fibroblast activation

Fibroblast activation refers to the process by which fibroblasts rapidly produce cytokines, chemokines, prostanoids, large amounts of ECM proteins (both constituents and degradation proteins) and express α -smooth-muscle actin in response to tissue injury (7, 17, 34). Activated fibroblasts are found in damaged, inflamed or healing tissue. These activated cells regulate the behaviour of leukocytes that are recruited to damaged tissue. CD40-CD40 ligand interactions is known to regulate this process by activating the nuclear factor (NF)- κ B family of transcription factors, leading to myofibroblast production of large amounts of IL-6, IL-8, cyclooxygenase-2 (COX-2) and hyaluronan

(35, 36). Cytokines and chemokines produced by fibroblasts appear to be specific to the type of injurious stimulus and the anatomical site of pathology (15).

3.0 The stroma in chronic inflammation

Chronic inflammation is characterised by its persistence, predilection for certain anatomical sites and propensity to be associated with significant disease related comorbidity including cancer, infection and diseases affecting the cardiovascular system and bone. Inflammation can only resolve if leukocyte recruitment, accumulation and survival is outweighed by leukocyte emigration and apoptosis at the site of inflammation. Why chronic inflammation fails to completely resolve, even after removal of injurious stimuli has, until relatively recently been poorly understood.

3.1 Accumulation of epigenetic changes and stability of the inflamed microenvironment

The structural composition of inflammatory sites at a microscopic level shows that the tissue microenvironment is defined largely by resident stromal cells such as fibroblasts (17). In chronic inflammation and during tissue fibrosis, fibroblasts maintain a state of permanent activation even once the initial stimulus has regressed. This activated phenotype persists until cells reach senescence (7). As with alterations in fibroblast phenotype by anatomical site, activated fibroblasts from diseased tissue (kidney, synovium, lung parenchyma) are also distinct in their phenotype and immune properties compared to fibroblasts from the same site in health (37-39). Furthermore, persistent activation of fibroblasts is stable; they maintain their distinctive phenotype even in tissue culture and in the absence of external stimulation and leukocytes (17). These findings indicate that permanently activated fibroblasts create inflamed tissue microenvironments that are intrinsically imprinted and stable (17). The molecular basis of persistent fibroblast activation in chronic inflammation is poorly understood. However recent evidence suggests that stable epigenetic modifications of the fibroblast genome (e.g. DNA hypomethylation) in response to inflammatory insults may be an important contributing factor (40, 41). Repeated episodes of acute, non-resolving inflammation may cause multiple epigenetic changes in the genome of the fibroblast, having a synergistic effect on promotion of stability of the activated, site-specific phenotype.

3.2 Recruitment of leukocytes to sites of chronic inflammation

Evidence from co-culture models suggests that physiological stromal tissue, which is not imprinted with an inflammatory phenotype, can become immunomodulatory, inhibiting and regulating leukocyte migration through ECs via IL-6 cytokine signalling (31). In contrast to fibroblasts from healthy tissue, rheumatoid arthritis synovial fibroblasts (RA-SFs) activate co-cultured ECs and support recruitment and migration of neutrophils and lymphocytes *in vitro* in the absence of external stimuli (32, 42-44). Adhesion of infiltrating leukocytes to stromal tissue appears to be mediated by fibroblast-derived chemokines (42). Neutrophil activation occurs when CXCR2 binds CXCL5, lymphocyte activation occurs when CXCR4 binds to CXCL12, also known as stromal derived factor-1 (SDF-1) (32, 42, 43). For both lymphocytes and neutrophils, recruitment is dependent on IL-6 generated in the co-culture period (32, 42, 43).

Along with stimulation of adhesion and leukocyte activation, inflammatory fibroblasts from the rheumatoid synovium have been shown to promote onward migration of T cells through the endothelial cell layer in co-culture models (44). When T cells are brought in close contact with RA-SFs that have been cultured with ECs, migration through cell layers occurs significantly more rapidly than when the same experiment is performed with dermal fibroblasts. This difference appears to be mediated by the increased production of CXCL12/SDF-1 by the RA-SFs (45).

3.3 Retention of lymphocytes

The process of generating an adaptive immune response requires well-coordinated and precise immune cell migration. Dendritic cells must sample antigen from the site of inflammation, then migrate to the draining lymph node to present the antigen to helper T cells. This process

requires specific, accurate and timely chemokine gradients to ensure that the immune cells interact at the right time, and in the right place. It has been suggested that activated fibroblasts may destabilise the physiological chemokine gradients underpinning this process through the upregulation and secretion of chemokines which promote retention of lymphocytes at a particular site. (47)

Leukocyte survival factors such as type 1 interferons, CXCR4 and its ligand CXCL12/SDF-1 contribute to the retention of lymphocytes as lymphoid aggregates in secondary lymphoid tissue (17, 57-59). SDF-1/CXCL12 is usually responsible for retention of lymphocytes within the bone marrow. Up-regulation of CXCR4 on infiltrating T lymphocytes by stromal derived TGF- β and expression of SDF-1 has been observed in inflamed synovium, intestine and skin (60, 61). It is also possible that fibroblasts imprinted with an inflammatory phenotype fail to switch off production of SDF-1 (17). These findings suggest that 'pro-retention' factors are important in the maintenance of the chronic inflammation; promoting retention of lymphocytes in an area of inflammation and inhibition of leukocyte migration minimises disruption. In other words the inflamed microenvironment becomes even more stable.

3.4 Tertiary lymphoid organs (TLOs)

The chemokines and adhesion molecules involved in recruitment of T cells to specific anatomical sites in health are well characterised. (46). Naïve T cells display a restricted pattern of recirculation, homing only to secondary lymphoid tissue using L-selectin, LFA-1 and the chemokine receptor CCR7. Memory T cells, in contrast, migrate to any anatomical site depending on the specific pattern of chemokines, receptors and adhesion molecules expressed (47).

As outlined above, activated fibroblasts undergo epigenetic and phenotypic changes in response to chronic inflammation. Activated rheumatoid synovial fibroblasts for example can resemble lymphoid fibroblasts when appropriately stimulated for long enough during episodes of acute inflammation (8, 33, 47, 48). When the stromal area postcode becomes sufficiently disordered in non-infectious/non-antigen specific chronic inflammation, fibroblasts and endothelial cells drive T-lymphocytes to accumulate at the area of disease and become organised into aggregates with structure reminiscent of lymph node structures, termed tertiary lymphoid organs (TLOs). (49).

TLOs are found in many chronic autoimmune disorders including rheumatoid arthritis, type 1 diabetes mellitus, Sjogren's syndrome, Hashimoto's thyroiditis and Crohn's disease (16). The mechanism for TLO formation has been explored in mouse models of diabetes where the expression of the homeostatic/lymphoid chemokine CXCL13 within pancreatic islets causes B cell recruitment and lymphoid neogenesis (50). CCL21, a chemokine specific to naïve lymphocytes, when ectopically expressed in transgenic mouse models, also leads to accumulation of T cell lymphoid infiltrates (51). More recent studies have shown that CXCL13 blockade disrupts B lymphocyte organization in tertiary lymphoid structures in non-obese diabetic mice (52). In the rheumatoid synovium, lymphoid-like fibroblasts produce CXCL13 and CCL19 driving lymphoid neogenesis (17, 45, 53). CCL19, VCAM-1 and ICAM-1 have been shown to play a role in TLO formation (54, 55).

Lymphoid-like fibroblasts also express Gp38 (podoplanin), a glycoprotein which is expressed by cells within areas of healthy, secondary lymphoid tissue (16). In non-lymphoid, physiological fibroblasts, gp38 is not expressed. Upon transformation of fibroblasts into a lymphoid like phenotype, gp38 and CD157 (BP-3) become expressed, leading to production of IL-7 and the lymphoid cytokines CXCL13 and CCL19 (17, 45, 53). Expression of gp38 has also been detected in various types of malignancy. In both malignancy and chronic inflammation, expression of gp38 corresponds with disease aggression and poor prognosis (16). Accordingly, it is important to note that the histological finding of TLOs in the synovium of patients with early RA is associated with severe disease progression and erosive disease (56).

3.5 Inhibition of leukocyte apoptosis

Resolution of an inflammatory response requires the removal of the majority of immune cells that were recruited and then proliferated at a site of inflammation. This process involves leukocyte emigration from the site of pathology and extensive apoptosis of activated T-cells (58). To maintain immunological memory, a few of these T cells must survive the wave of tissue apoptosis. The process of T-cell apoptosis at sites of pathology is therefore tightly regulated. There is now strong evidence that this process is stromal cell mediated (58).

In order to resolve an acute episode of inflammation, active Fas-induced apoptosis of leukocytes occurs, limiting the extent of tissue damage and acting as the trigger for the resolution phase (62). This process acts in synergy with apoptosis resulting from cytokine deprivation (63).

In T-cells, IL-2 (predominantly), IL-4, IL-7, IL-9 and IL-15 use a shared receptor signalling component which forms the γ chain of the IL-2 receptor (58, 64, 65). When these cytokines are withdrawn, T cell apoptosis occurs readily (66). This cytokine-mediated inhibition of apoptosis occurs via upregulation of the genes BCL-2 and Bcl-x_L and increased T-cell proliferation (64). Cytokine-deprived T cells in rheumatoid arthritis however may be rescued from apoptosis via interaction with RA-SFs (64, 67). This process however, does not involve increased expression of BCL-2 or T-cell proliferation (66), suggesting that fibroblast mediated inhibition of T-cell apoptosis occurs via a mechanism which is distinct from cytokine deprivation. The NF- κ B family of transcription factors which is heavily upregulated in the rheumatoid synovium has been implicated in the regulation of bcl-2 regulated apoptosis (68). Type one interferons, particularly IFN- β , are the predominant factors influencing stromal mediated inhibition of apoptosis (58). Our group has proposed therefore, that RA-SF mediated blockade of lymphocyte death through upregulation of aberrant 'pro-survival' cytokines/chemokines is a major contributing factor to the persistence of T cell infiltration despite low levels of T-cell cytokines in the chronically inflamed rheumatoid synovium (64).

B-cell survival depends on fibroblast production of IL-6 and expression of CXCL12 (69). Expression of the type II membrane proteins; BAFF (B cell activating factor) and APRIL (a proliferation inducing ligand) by RA-SFs have been shown to mediate B cell survival and function within the RA synovium (70).

3.6 Tissue destruction in RA

RA-synovial fibroblasts (RA-SFs) within the lining layer of the synovial joint cartilage are known to contribute to joint destruction and bony erosions through expression of degradation enzymes such as MMPs (71). MMP-2, MMP-9 and MMP-13 have been specifically implicated (72). MMP-9 is also upregulated by CXCL12 (SDF-1) (73). Fibroblast activation protein (FAP) is expressed at high levels in the lining layer of the synovium, and is co-localised with MMP-13 within the rheumatoid synovium (74). Cathepsins, a major group of proteases, which are involved in joint destruction, have also been shown to be upregulated in the rheumatoid synovium (75). When RA-SFs are transfected with the *ras* proto-oncogene, cathepsin L is upregulated (76). The pro-inflammatory cytokines IL-1, IL-6 and TNF α also stimulate the production of cathepsins; an effect mediated by RA-SFs (77).

3.7 p21 cyclin dependent kinase inhibitors (CDKi)

Cyclin dependent kinases (CDKs) are primarily responsible for regulation of the cell cycle. CDKi overexpression induces cell cycle arrest (78). p21, a CDKi has been shown to inhibit arthritis development in mouse models (79). The expression of p21 by synovial fibroblasts is reduced in isolated RA-SFs compared to OA synovial fibroblasts (80). In p21-deficient synovial fibroblasts in mice, a 100-fold increase in IL-6 protein and enhanced IL-6 mRNA is seen (80). Overexpression of MMPs and cathepsins has also been noted in p21 deficient mice (81) thus suggesting a protective, anti-inflammatory role for p21 CDKi in RA.

Table 2: Fibroblast mediated persistence of chronic inflammation

Recruitment	TLO formation	Retention	Reduced emigration	Reduced apoptosis
IL-1	gp38	IFN-1	CXCL12 (SDF-1)	Bcl-x _L
IL-6	CD157 (BP-3)	IL-15	CCL21	BCI-2*
TNF α	IL-7	BAFF		IFN- β
COX-2	CXCL13 (BLC)	CXCL12 (SDF-1)		NF- κ B
CXCL8/IL-8	CCL19	CXCR4		IL-2
CCL5	CCL21 (SLC)	TGF- β		IL-4
CXCL1	ICAM-1			IL-15
CXCR2	VCAM-1			BAFF [^]
CXCL5				APRIL [^]
CXCL12 (SDF-1)				IL-6 [^]
				CXCL12 [^] (SDF-1)

*Low levels of BCI-2 are observed during fibroblast mediated inhibition of lymphocyte apoptosis. All other molecules are up regulated by fibroblasts to modulate persisting inflammation. [^]Increase survival of B-cells.

3.8 The synovium in patients with early rheumatoid arthritis

The model of persistent inflammation in RA proposed above requires the presence of lymphoid aggregates and a hyperplastic synovium. These features are rare in patients with active synovitis in the early stages of the disease. It is therefore likely that the pathological mechanisms underpinning disease initiation are distinct from those causing disease persistence. Additionally the observation that spontaneous remission is unusual when an inflammatory arthritis has persisted for longer than 6 months (82) suggests that the stromal-mediated processes, as outlined above, are well established by this time. In support of this idea is the finding that the cytokine/chemokine profile in the synovial fluid of patients with early (less than 12 weeks onset) synovitis who were likely to subsequently progress to persistent RA was distinct from synovial fluid from the same patient cohort who were unlikely to progress to established RA (83). The early synovial cytokine/chemokine profile from patients who were likely to develop RA demonstrated high levels of T-cell, macrophage and stromal derived cytokines (IL-2, IL-4, IL-13, IL-17, IL-1, IL-15, bFGF and EGF). This profile is transient and is not present in the synovium of patients with persistent disease. These findings suggest that the type of immune response that leads to RA involves stromal components and Th2 cytokines and is likely to influence the biology of the tissue microenvironment required for persistent disease (83).

4.0 Biological agents in RA that target the stroma

Cytokine inhibition using biological therapies has revolutionized the management of rheumatoid arthritis in recent years. Despite the successes of this approach, we are still unable to cure the disease. Most therapies share a maximum therapeutic response rate of around 20% achieving an ACR 70 response (15). As we have outlined, stromal derived cytokines such as IL-1, IL-6 and TNF α are intermediate effectors of the autoimmune response in RA. It is therefore not surprising that their blockade dampens inflammation as opposed to eliminating it. In this review we have outlined that stromal elements, in particular fibroblasts, act upstream of these cytokines and are

responsible for the persistence of chronic inflammation in rheumatoid arthritis. There are however, at present, no therapeutic agents available which directly target the fibroblasts, nor are there agents which target the potent fibroblast-effectors modulating persistence (TLO formation, pro-retention factors and pro-survival factors). However, conventional biologics do have effects on the stroma and their cellular products. For example Tocilizumab, which targets IL-6, has anti stromal effects. In active rheumatoid arthritis, IL-6 is found in abundance in both the synovium and the circulation (32). Within the inflamed synovium, IL-6 is produced by activated synovial fibroblasts and is pro-inflammatory. Downstream pro-inflammatory effects include stimulation of differentiation of B cells into immunoglobulin secreting plasma cells, activation of T cells (84), promotion of B-cell survival (69), and the recruitment of neutrophils and lymphocytes (32, 42, 43). IL-6 also contributes to joint destruction by interaction with cathepsins (77).

4.1 Possible future stromal targets

Cellular MAPK and NF- κ B pathways are heavily implicated in the inhibition of T cell apoptosis in the rheumatoid synovium. These pathways however are generic; involved in directing the cellular responses in numerous cellular systems. More specific MAPK effectors are therefore being targeted, of which p38 MAPKinases which share similarities with the janus kinase pathway are known to impact on synovial fibroblast biology (86).

Fibroblasts express a number of sensitive, but non-specific markers (Table 1). Targeting pathogenic fibroblasts alone, without collateral damage, is therefore a challenge. Research is currently underway to identify pathogenic fibroblast markers. Cadherin-11 is both a sensitive fibroblast marker and is relatively specific to the inflammatory synovium in mouse models of arthritis. Cadherin-11 promotes invasive behaviour of RA-SFs (87). Its high specificity to pathological states makes it an attractive potential target for further investigation. CD248 is a similar fibroblast target, which has a role in adhesion and its blockade in mouse models of arthritis has been promising (88). RA-SFs express low levels of the p21 CDKi, translating to a pro-inflammatory effect mediated by IL-6 (80). Adenovirus mediated p21 gene transfer to RA-SFs is currently under investigated to treat RA in early clinical trials. Finally, blockade of CXCR4, the receptor for CXCL12/SDF-1 using the antagonist AMD3100 in mouse models of arthritis has shown significant reduction in disease severity (89) In human trials however, its use has been abandoned due to its adverse effects including bone marrow suppression (15).

5.0 Conclusions

Fibroblasts play a role in regulating the accumulation of leucocytes in inflamed tissues. In response to recurrent inflammatory stimuli, fibroblasts undergo phenotypic alterations, which alter their pathogenic potential. These alterations are very specific and contextual. In the case of inflammatory states such as rheumatoid arthritis, the fibroblast phenotype changes to one which behaves like a lymphoid fibroblast; supporting leukocyte adhesion and migration, lymphoid neogenesis, retention of leukocytes and inhibition of leukocyte apoptosis. All of these promote persistent and stable inflammation within the synovium.

Currently available biologic agents for use in rheumatoid arthritis do not directly target the fibroblast but do target their cell products (eg IL-6), which mediate persistence of inflammation. As such, current available therapies for rheumatoid arthritis do not truly target the persistence of inflammation. Novel therapeutic agents directed at tissue resident stromal cells and which aid their depletion offers an exciting new avenue of research. These agents may turn out to be the key to the cure of persistent inflammatory disorders such as rheumatoid arthritis.

6.0 Practice points

- IL-1, IL-6 and TNF α blockade is useful to suppress persistent disease activity in patients with rheumatoid arthritis
- Future targeting of stromal pathways involved in the switch of acute to chronic inflammation (gp38, CXCR4) may be useful to prevent persistent synovitis and joint destruction in patients with early (<12 weeks) synovitis

7.0 Research Agenda

- Continued search for more specific fibroblast markers
- Data on the use of novel fibroblast targeted agents/genetic therapies for use in chronic inflammatory conditions
- Targeting epigenetic modifications to alter RA-SF phenotype
- Development of a unified understanding of the immunopathogenesis of rheumatoid arthritis, with particular focus on the immune mechanisms underpinning the switch from resolving to persistent inflammation to guide research into more novel targets that may be used to prevent rheumatoid arthritis in patients with early synovitis who are at significant risk of developing persistent disease.

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